Dual FLT3/TOPK inhibitor with activity against FLT3-ITD secondary mutations potently inhibits AML cell lines

Supplemental Experimental procedures

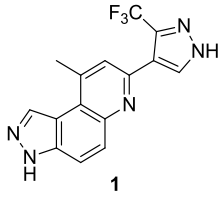
Chemistry

General Considerations Unless noted otherwise, all reagents and solvents were purchased from commercial sources and used as received. All reactions were performed in a screw-cap sealed vials. The ^{1}H and ^{13}C NMR spectra were obtained in CD_3OD or $(CD_3)_2SO$ as solvent using a 500 MHz spectrometer with Me_4Si as an internal standard. Chemical shifts are reported in parts per million (δ) and are calibrated using residual undeuterated solvent as an internal reference. Data for 1H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or combinations thereof. High resolution mass spectra (HRMS) were obtained using electron spray ionization (ESI) technique and as TOF mass analyzer. New compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS data. Substrate 3-(Trifluoromethyl)-1H-pyrazole-4-carbaldehyde and 5-amino indazole were purchased from 1Chem (95% purity) and Ark Pharm (98% purity) respectively.

General procedure for the multicomponent reaction:

A mixture of amine (1 mmol) and aldehyde (1 mmol) in 4 mL of absolute ethanol was refluxed for 2 h followed by addition of cyclic ketone or acetone (2.1 mmol) to the reaction mixture. A catalytic amount of conc. hydrochloric acid was added and the reaction was continued to reflux for 6-12 h. Reaction mixture was dissolved in ethylacetate (50 mL), washed with sodium bicarbonate and brine solution (20 mL X 2). The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and purified by silica gel chromatography (dichloromethane:methanol (99:01 to 80:20) to give the desired cyclized compound.

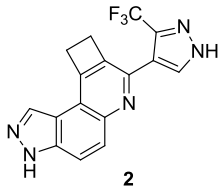
9-Methyl-7-(3-(trifluoromethyl)-1*H*-pyrazol-4-yl)-3*H*-pyrazolo[4,3-*f*]quinolone (1)



1

Off-white solid (136 mg, 43%). ^{1}H NMR (500 MHz, MeOD) δ 8.51 (s, 1H), 8.29 – 8.21 (m, 1H), 7.91 – 7.81 (m, 2H), 7.62 (s, 1H), 2.89 (s, 3H);^{13}C NMR (126 MHz, MeOD) δ 147.45, 145.74, 144.37, 139.03, 138.40, 135.27, 130.97, 128.87, 125.13 (*J* = 269.64 Hz), 122.50, 121.51, 120.86, 116.35, 114.74, 21.45; HRMS (ESI) m/z calcd for $C_{15}H_{11}F_3N_5$ $[M + H]^+$ 318.0967, found 318.0971.

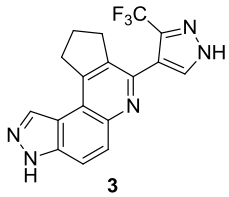
7-(3-(Trifluoromethyl)-1H-pyrazol-4-yl)-8,9-dihydro-3H-cyclobuta[c]pyrazolo[4,3-*f*]quinolone (2)



2

Off-white solid (49 mg, 15%). ^{1}H NMR (500 MHz, MeOD) δ 8.69 (d, *J* = 0.9 Hz, 1H), 8.53 (d, *J* = 0.9 Hz, 1H), 8.32 (dd, *J* = 9.4, 1.0 Hz, 1H), 8.11 (d, *J* = 9.4 Hz, 1H), 4.01 – 3.93 (m, 2H), 3.72 (t, *J* = 4.0 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 161.67, 140.75, 139.64, 137.73, 136.67, 133.55, 133.12, 122.29 (q, *J* = 268.38 Hz), 121.46, 119.65, 119.42, 113.74, 108.80, 30.70, 29.72; HRMS (ESI) m/z calcd for $C_{16}H_{11}F_3N_5$ $[M + H]^+$ 330.0967, found 330.0970.

7-(3-(Trifluoromethyl)-1*H*-pyrazol-4-yl)-3,8,9,10-tetrahydrocyclopenta[*c*]pyrazolo[4,3-*f*]quinolone (3)

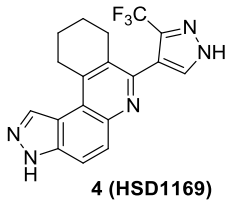


3

Off-white solid (185 mg, 54%). ^{1}H NMR (500 MHz, MeOD) δ 8.76 (s, 1H), 8.44 (s, 1H), 8.33 (d, *J* = 9.3 Hz, 1H), 8.05 (d, *J* = 9.3 Hz, 1H), 3.86 (t, *J* = 7.7 Hz, 2H), 3.21 (t, *J* = 7.7 Hz, 2H),

2.57– 2.53 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ 160.67, 141.10, 140.00, 138.86, 136.50, 133.37, 132.59, 122.32(q, *J* = 269.64 Hz), 121.78, 121.15, 118.59, 115.19, 110.20, 35.03, 30.90, 23.82; HRMS (ESI) m/z calcd for $C_{17}H_{13}F_3N_5$ $[M + H]^+$ 334.1123, found 334.1121.

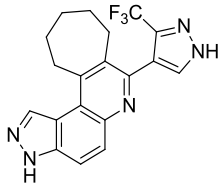
7-(3-(Trifluoromethyl)-1H-pyrazol-4-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine (4)



4 (HSD1169)

Off-white solid (161 mg, 45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.22 (s, 1H), 7.85 (d, *J* = 9.1 Hz, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 3.26 (t, *J* = 6.4 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 2H), 1.98 – 1.86 (m, 2H), 1.80 – 1.69 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 148.52, 143.61, 141.99, 139.65, 139.37(q, *J* = 35.28Hz), 135.14, 131.28, 130.36, 129.53, 123.56(q, *J* = 269.64Hz), 121.99, 119.73, 116.21, 115.24, 29.48, 28.17, 22.51, 22.29; HRMS (ESI) m/z calcd for $C_{18}H_{15}F_3N_5$ $[M + H]^+$ 358.1280, found 358.1286.

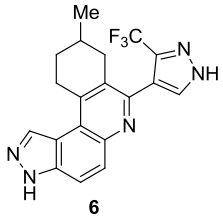
7-(3-(Trifluoromethyl)-1*H*-pyrazol-4-yl)-3,8,9,10,11,12hexahydrocyclohepta[*c*]pyrazolo[4,3-*f*]quinolone (5)



5

Off-white solid (185 mg, 50%). ^{1}H NMR (500 MHz, MeOD) δ 8.98 – 8.87 (m, 1H), 8.37 – 8.27 (m, 2H), 7.99 (d, *J* = 9.2 Hz, 1H), 3.86 (d, *J* = 5.3 Hz, 2H), 3.17 – 3.04 (m, 2H), 2.05 – 2.03 (m, 4H), 1.78 – 1.65 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ 160.75, 140.27, 136.43, 133.66, 132.53, 123.83, 122.46, 122.13, 121.07, 120.17(q, *J* = 269.64 Hz), 118.79, 114.94, 114.65, 110.56, 110.56, 32.30, 30.64, 29.52, 25.85, 23.75; HRMS (ESI) m/z calcd for $C_{19}H_{17}F_3N_5$ $[M + H]^+$ 372.1436, found 372.1429.

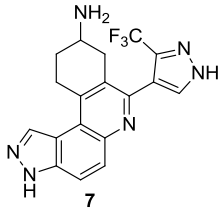
9-Methyl-7-(3-(trifluoromethyl)-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (6)



6

Off-white solid (178 mg, 48%).[1]H NMR (500 MHz, MeOD) δ 8.60 (s, 1H), 7.97 (d, *J* = 1.1 Hz, 1H), 7.86 (s, 2H), 3.56 – 3.48 (m, 1H), 3.39 – 3.32 (m, 1H), 2.77 – 2.70 (m, 1H), 2.38 – 2.28 (m, 1H), 2.27 – 2.18 (m, 1H), 1.97 – 1.85 (m, 1H), 1.66 – 1.53 (m, 1H), 1.09 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ 148.72, 143.06, 142.82, 139.74 (*J* = 28.98 Hz) 134.66, 131.35, 131.12, 131.01, 128.37, 123.01, 122.23, 120.84 (*J* = 269.64 Hz), 115.93, 114.67, 36.13, 30.14, 29.44, 28.15, 20.47; HRMS (ESI) m/z calcd for $C_{19}H_{17}F_3N_5$ $[M + H]^+$ 372.1436, found 372.1440.

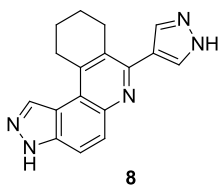
7-(3-(Trifluoromethyl)-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-9-amine (7)



7

Off-white solid (137 mg, 37%). [1]H NMR (500 MHz, MeOD) δ 8.57 (d, *J* = 7.7 Hz, 1H), 8.03 – 8.00 (m, 1H), 7.87 – 7.83 (m, 2H), 3.92 – 3.82 (m, 1H), 3.65 – 3.53 (m, 1H), 3.49 – 3.38 (m, 1H), 2.81 – 2.65 (m, 2H), 2.25 – 2.17 (m, 1H), 2.08 – 1.96 (m, 1H); ^{13}C NMR (126 MHz, MeOD) δ 148.28, 143.27, 142.44, 142.22, 139.73, 134.64, 130.59, 129.31, 128.37, 122.83(q, *J*= 269.64 Hz), 122.00, 118.74, 115.82, 114.97, 46.06, 36.25, 30.30, 28.58; HRMS (ESI) m/z calcd for $C_{18}H_{16}F_3N_6$ $[M + H]^+$ 373.1389, found 373.1393.

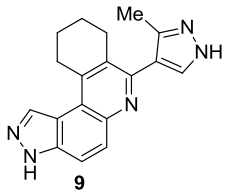
7-(1*H*-Pyrazol-4-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine



8

White solid (220 mg, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.14 (s, 2H), 7.79 (d, J = 5.4 Hz, 2H), 3.28 (d, J = 6.7 Hz, 2H), 2.99 (t, J = 6.1 Hz, 2H), 1.98 (ddt, J = 9.0, 6.4, 3.2 Hz, 2H), 1.84 – 1.82 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 149.72, 143.79, 142.11, 138.53, 136.11, 129.61, 128.83, 121.65, 121.03, 116.45, 114.22, 29.85, 28.67, 22.64, 22.48; HRMS (ESI) m/z calcd for $C_{17}H_{16}N_5$ $[M + H]^+$ 290.1406, found 290.1399.

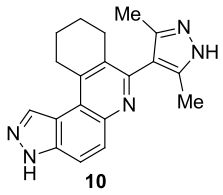
7-(3-Methyl-1H-pyrazol-4-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine (9)



9

Off-white solid (179 mg, 59%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.84 – 7.74 (m, 3H), 3.33 (s, 3H), 2.85 (t, J = 6.1 Hz, 2H), 2.33 (s, 2H), 2.00 – 1.97 (m, 2H), 1.81 – 1.77 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 151.10, 143.71, 141.83, 138.63, 136.21, 130.12, 129.59, 121.02, 118.17, 116.48, 114.04, 29.70, 28.62, 22.63; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_5$ $[M + H]^+$ 304.1562, found 304.1552.

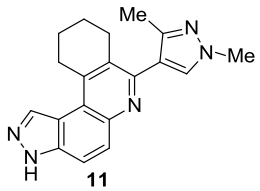
7-(3,5-Dimethyl-1H-pyrazol-4-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine (10)



10

Off-white solid (193 mg, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 – 8.53 (m, 1H), 7.82 (dd, J = 9.1, 0.9 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 3.30 (t, J = 6.5 Hz, 2H), 2.56 (t, J = 6.2 Hz, 2H), 2.00 (s, 8H), 1.76 (qd, J = 8.6, 7.2, 4.1 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 151.69, 144.07, 141.77, 139.31, 135.31, 131.30, 129.63, 121.60, 117.69, 116.32, 114.93, 29.51, 27.72, 22.74, 22.45; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_5$ $[M + H]^+$ 318.1719, found 318.1710.

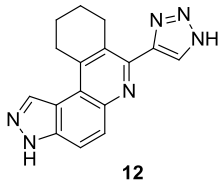
7-(1,3-Dimethyl-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (11)



11

Off-white solid (180 mg, 57%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.92 (s, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 3.82 (s, 3H), 3.27 (t, J = 6.5 Hz, 2H), 2.81 (t, J = 6.2 Hz, 2H), 2.26 (s, 3H), 2.01 – 1.88 (m, 2H), 1.82 – 1.67 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 150.70, 147.24, 143.71, 141.82, 139.32, 135.07, 131.72, 129.87, 129.57, 121.05, 118.85, 116.35, 114.88, 38.70, 29.70, 28.60, 22.60, 13.53; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_5$ $[M + H]^+$ 318.1719, found 318.1709.

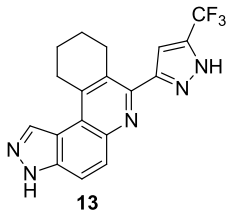
7-(1*H*-1,2,3-Triazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (12)



12

Off-white solid (159 mg, 55%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.31 (d, J = 1.9 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.82 (dd, J = 9.1, 1.9 Hz, 1H), 3.30 (d, J = 6.6 Hz, 2H), 3.12 (d, J = 6.4 Hz, 2H), 2.05 – 1.94 (m, 2H), 1.86 – 1.81 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 146.46, 143.65, 142.93, 131.52, 129.80, 129.48, 122.18, 116.16, 115.49, 29.87, 28.27, 22.37; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_6$ $[M + H]^+$ 291.1358, found 291.1349.

7-(5-(Trifluoromethyl)-1H-pyrazol-3-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine (13)

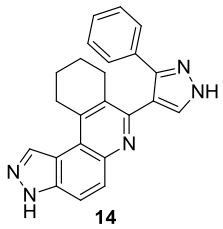


13

Off-white solid (150 mg, 42%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.17 (s, 1H), 3.33 – 3.28 (m, 2H), 3.00 (t, J = 6.2 Hz, 2H), 2.02 – 1.95 (m, 2H), 1.88 –

1.80 (m, 2H);[13]C NMR (126 MHz, DMSO) δ 144.59, 143.32, 142.97, 142.16, (*J* = 36.83 Hz), 138.87, 136.55, 129.44, 125.49 (*J* = 270.51 Hz), 122.50, 116.27, 115.12, 104.88, 29.83, 27.83, 22.26, 22.22; HRMS (ESI) m/z calcd for $C_{18}H_{15}F_3N_5$ $[M + H]^+$ 358.1280, found 358.1282.

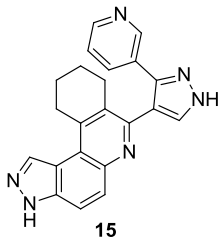
7-(3-Phenyl-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (14)



14

Off-white solid (168 mg, 45%). [1]H NMR (500 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.27 (d, *J* = 9.1 Hz, 2H), 8.17 (d, *J* = 9.2 Hz, 1H), 7.35 – 7.22 (m, 5H), 3.51 – 3.43 (m, 2H), 2.55 – 2.49 (m, 2H), 1.95 – 1.82 (m, 2H), 1.72 – 1.61 (m, 2H); [13]C NMR (126 MHz, DMSO) δ 145.52, 135.52, 132.94, 129.41, 128.96, 128.85, 128.19, 126.99, 123.39, 120.03, 114.91, 109.66, 30.80, 27.32, 21.75, 21.27; HRMS (ESI) m/z calcd for $C_{23}H_{20}N_5$ $[M + H]^+$ 366.1719, found 366.1726.

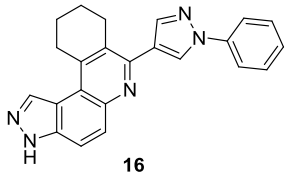
7-(3-(Pyridin-3-yl)-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (15)



15

Off-white solid (172 mg, 47 [1]H NMR (500 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.48 (s, 1H), 8.40 – 8.31 (m, 1H), 7.99 (s, 1H), 7.83 (d, *J* = 9.1 Hz, 1H), 7.78 – 7.63 (m, 2H), 7.25 (dd, *J* = 8.1, 4.8 Hz, 1H), 3.34 – 3.22 (m, 2H), 2.58 (t, *J* = 6.2 Hz, 2H), 1.96 – 1.83 (m, 2H), 1.73 – 1.65 (m, 2H); [13]C NMR (126 MHz, DMSO) δ 150.81, 148.62, 147.71, 143.91, 143.51, 142.22, 140.11, 135.03, 134.04, 133.41, 130.51, 129.58, 129.11, 123.91, 121.95, 119.13, 116.29, 115.55, 29.57, 28.27, 22.59, 22.35; HRMS (ESI) m/z calcd for $C_{22}H_{19}N_6$ $[M + H]^+$ 367.1671, found 367.1661.

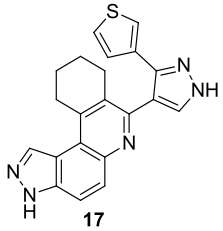
7-(1-Phenyl-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (16)



16

Off-white solid (186 mg, 51%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.55 (s, 1H), 8.29 (d, *J* = 1.7 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 2H), 7.88 – 7.81 (m, 2H), 7.55 – 7.49 (m, 2H), 7.36 – 7.30 (m, 1H), 3.31 (t, *J* = 6.4 Hz, 2H), 2.58 (t, *J* = 6.1 Hz, 2H), 1.94 – 1.85 (m, 2H), 1.74 – 1.65 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 150.93, 143.68, 142.18, 138.73, 136.97, 136.67, 136.31, 131.22, 130.28, 129.55, 127.67, 125.20, 123.94, 122.00, 118.02, 116.54, 114.31, 29.56, 28.10, 22.55, 22.32; HRMS (ESI) m/z calcd for $C_{23}H_{20}N_5$ $[M + H]^+$ 366.1719, found 366.1722.

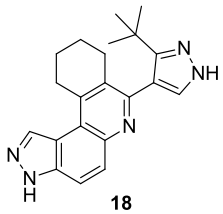
7-(3-(Thiophen-3-yl)-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridine (17)



17

Off-white solid (177 mg, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.03 – 7.91 (m, 1H), 7.88 – 7.76 (m, 2H), 7.39 – 7.23 (m, 1H), 6.80 (dt, *J* = 6.1, 3.0 Hz, 1H), 6.62 (d, *J* = 3.2 Hz, 1H), 3.35 – 3.32 (m, 2H), 2.70 – 2.54 (m, 2H), 1.98 – 1.84 (m, 2H), 1.79 – 1.63 (m, 2H).; ^{13}C NMR (126 MHz, DMSO) δ 148.63, 143.82, 142.45, 142.18, 141.18, 140.01, 139.07, 130.03, 129.57, 129.19, 127.95, 126.90, 124.53, 121.44, 119.01, 118.46, 116.29, 29.86, 28.53, 22 .57, 22.45; HRMS (ESI) m/z calcd for $C_{21}H_{18}N_5S$ $[M + H]^+$ 372.1283, found 372.1274.

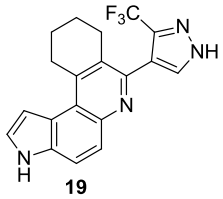
7-(3-(*tert*-Butyl)-1H-pyrazol-4-yl)-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-*a*]phenanthridine (18)



18

Off-white solid (176 mg, 51%).[1]H NMR (500 MHz, MeOD) δ 8.55 (s, 1H), 7.88 – 7.79 (m, 2H), 7.53 (s, 1H), 3.37 – 3.31 (m, 2H), 2.67 (t, *J* = 6.2 Hz, 2H), 2.08 – 1.97 (m, 2H), 1.90 – 1.81 (m, 2H), 1.17 (s, 9H); [13]C NMR (126 MHz, MeOD) δ 152.31, 143.08, 142.67, 138.68, 135.79, 131.90, 128.29, 122.19, 116.70, 116.13, 113.68, 32.29, 32.24, 29.44, 29.30, 28.47, 22.15, 21.89; ; HRMS (ESI) m/z calcd for $C_{21}H_{24}N_5$ $[M + H]^+$ 346.2032, found 346.2021.

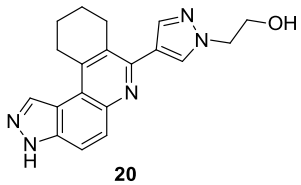
7-(3-(Trifluoromethyl)-1*H*-pyrazol-4-yl)-8,9,10,11-tetrahydro-3*H*-pyrrolo[3,2-*a*]phenanthridine (19)



19

Off-white solid (135 mg, 38%).[1]H NMR (500 MHz, MeOD) δ 7.92 (s, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 7.71 – 7.65 (m, 1H), 7.45 – 7.40 (m, 1H), 7.18 (d, *J* = 3.2 Hz, 1H), 3.46 (t, *J* = 6.4 Hz, 2H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.00 – 1.86 (m, 2H), 1.85– 1.86 (qd, *J* = 6.5, 3.2 Hz, 2H); [13]C NMR (126 MHz, MeOD) δ 146.73, 143.44, 142.52, 133.34, 130.08, 129.00, 123.12, 122.85, 121.93, 120.17, 119.38, 116.04, 105.77, 29.74, 27.88, 22.38, 21.90; HRMS (ESI) m/z calcd for $C_{19}H_{16}F_3N_4$ $[M + H]^+$ 357.1327, found 357.1330.

2-(4-(8,9,10,11-Tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)-1*H*-pyrazol-1-yl)ethan-1-ol (20)

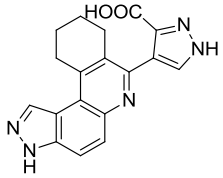


20

Off-white solid (126 mg, 38%). [1]H NMR (500 MHz, MeOD) δ 8.51 (s, 1H), 8.12 (s, 1H), 7.99 (s, 1H), 7.87 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 4.34 (t, *J* = 5.4 Hz, 2H), 3.97 (t, *J* = 5.4

Hz, 2H), 3.35 – 3.32 (m, 2H), 3.00 (t, *J* = 6.2 Hz, 2H), 2.10 – 2.03 (m, 2H), 1.95 – 1.86 (m, 2H). ^{13}C NMR (126 MHz, MeOD) δ 149.36, 143.28, 143.07, 139.58, 138.49, 135.72, 131.37, 129.45, 128.70, 121.71, 121.43, 116.16, 113.49, 60.48, 54.17, 29.73, 28.38, 22.12, 22.03; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_5O$ $[M + H]^+$ 334.1668, found 334.1666.

4-(8,9,10,11-Tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)-1*H*-pyrazole-3-carboxylic acid (21)



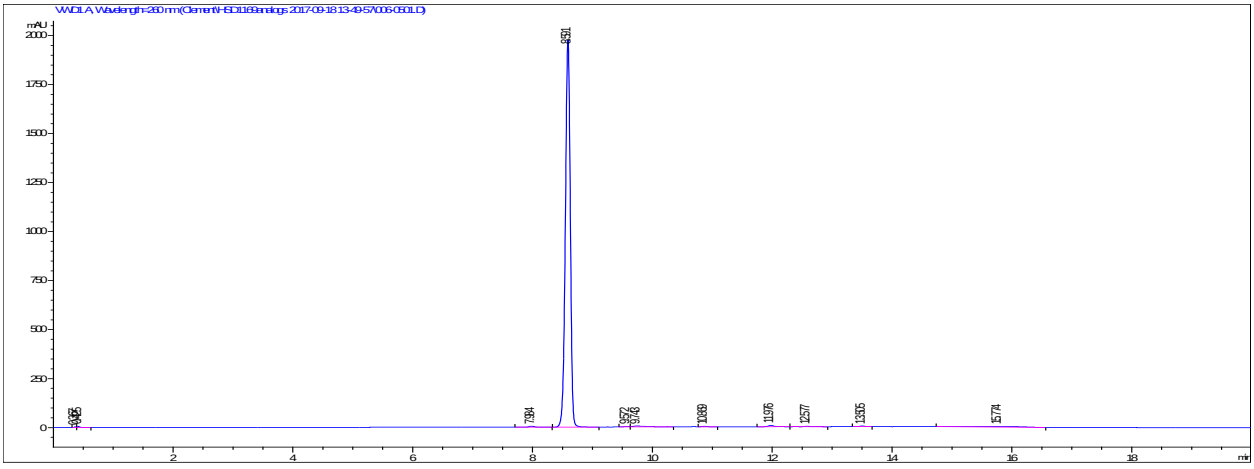
21

Off-white solid (167 mg, 50%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.25 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.76 (d, *J* = 9.4 Hz, 1H), 3.30 (t, *J* = 6.5 Hz, 2H), 2.93 – 2.78 (m, 2H), 2.03 – 1.91 (m, 2H), 1.83 – 1.74 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 162.03, 148.76, 145.57, 139.73, 137.07, 136.25, 131.58, 128.69, 123.61, 122.18, 121.89, 116.71, 115.13, 30.40, 28.34, 21.77. HRMS (ESI) m/z calcd for $C_{18}H_{16}N_5O_2$ $[M + H]^+$ 334.1304, found 334.1308.

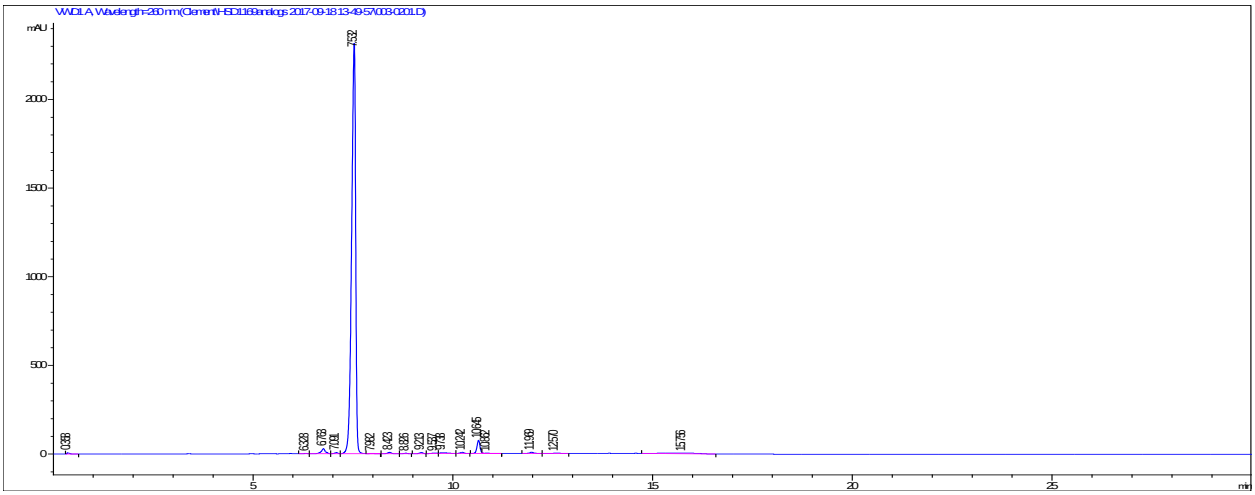
HPLC Data

Condition: Agilent Eclipse plus C18 coloumn, 3.5 μm, 4.6×100 mm, 0→4 min, 50% B, 5→14 min, 100% B, 15→20 min, 100% A (A: 0.1% NH_4OH in H_2O, B: MeOH), 55 °C.

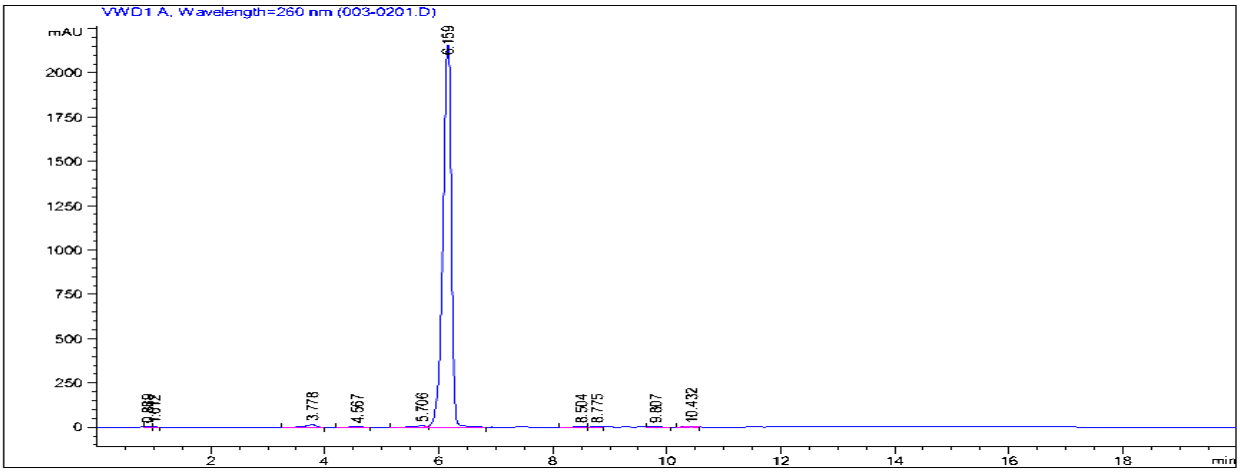
HPLC of compound HSD1169



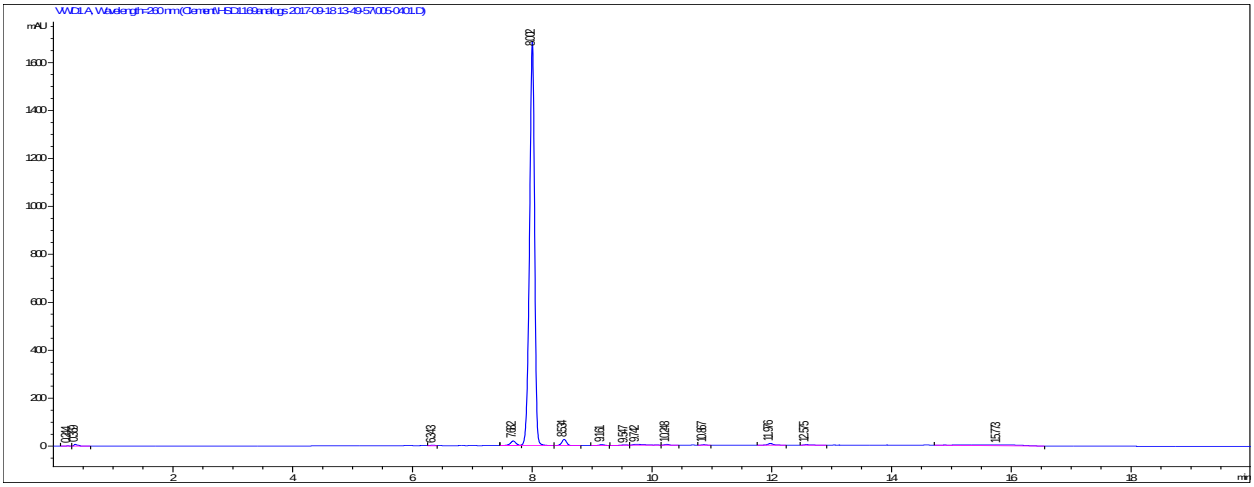
HPLC of compound 1



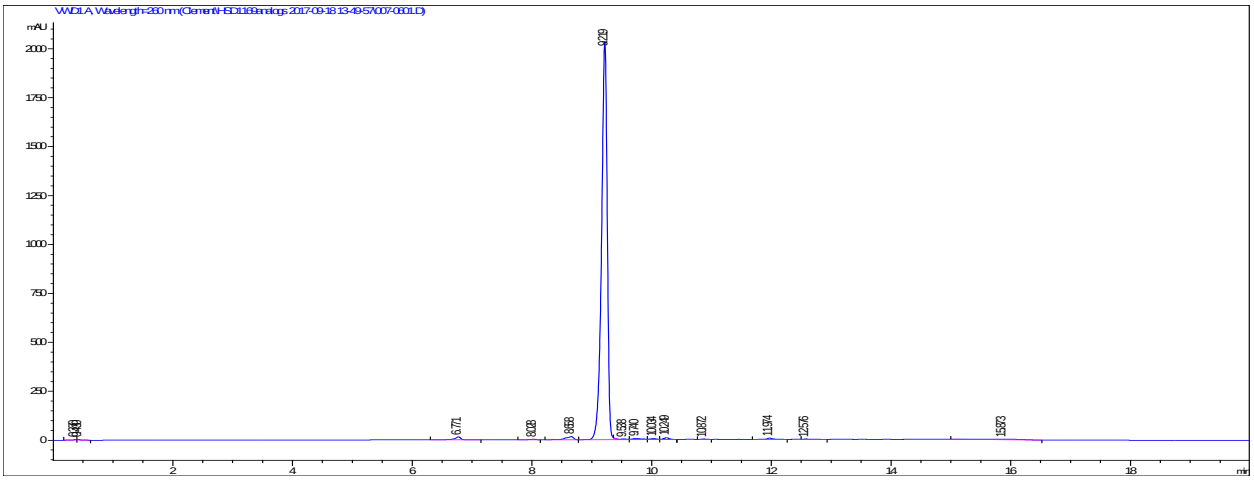
HPLC of compound 2



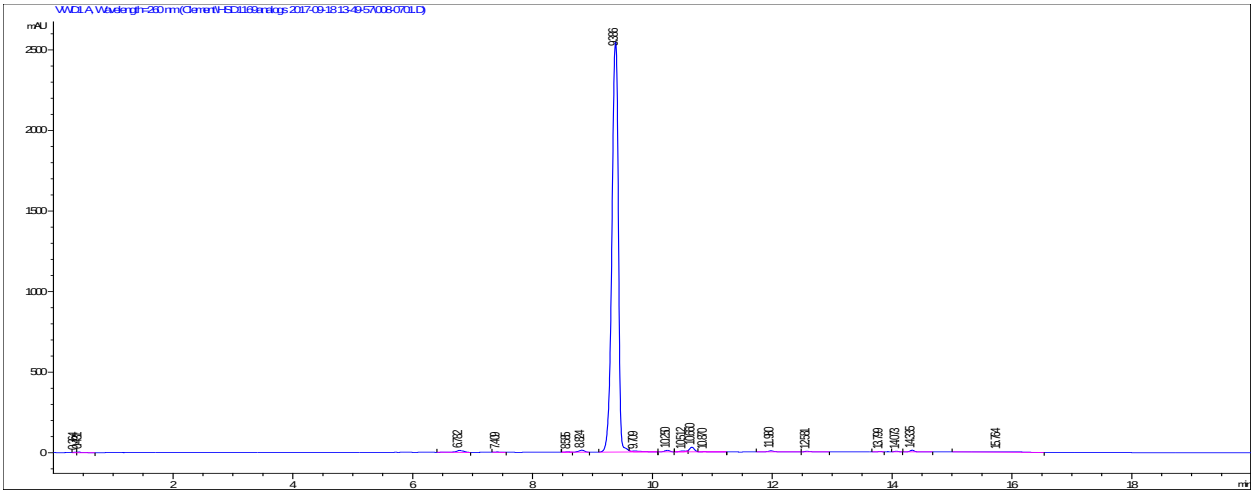
HPLC of compound 3



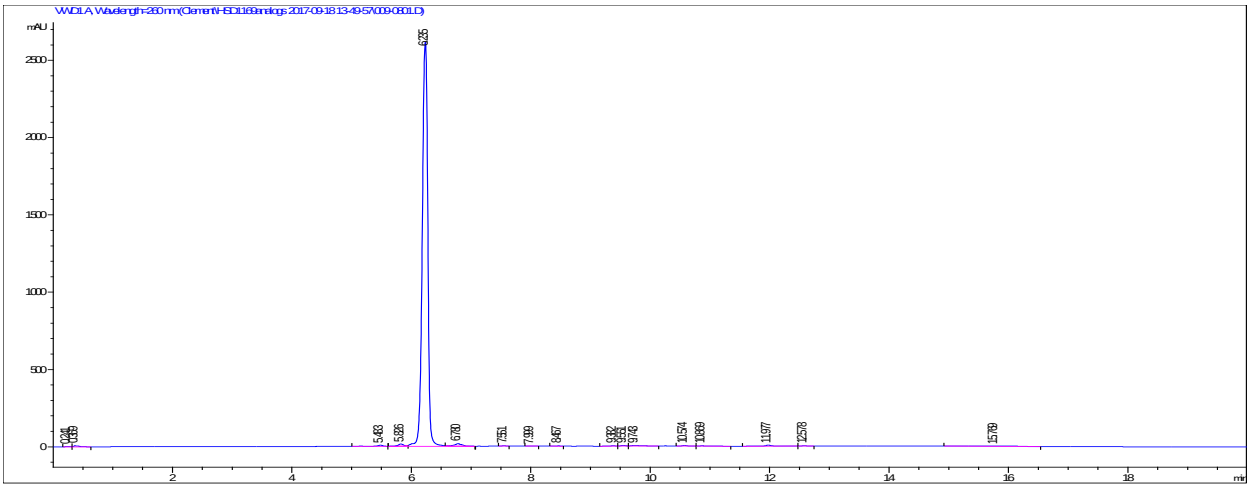
HPLC of compound 5



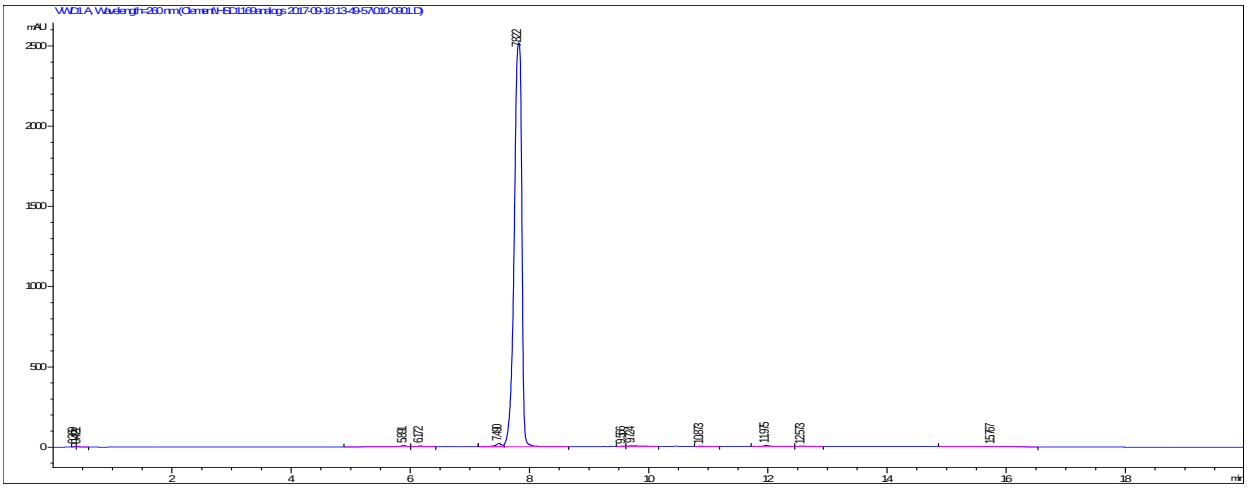
HPLC of compound 6



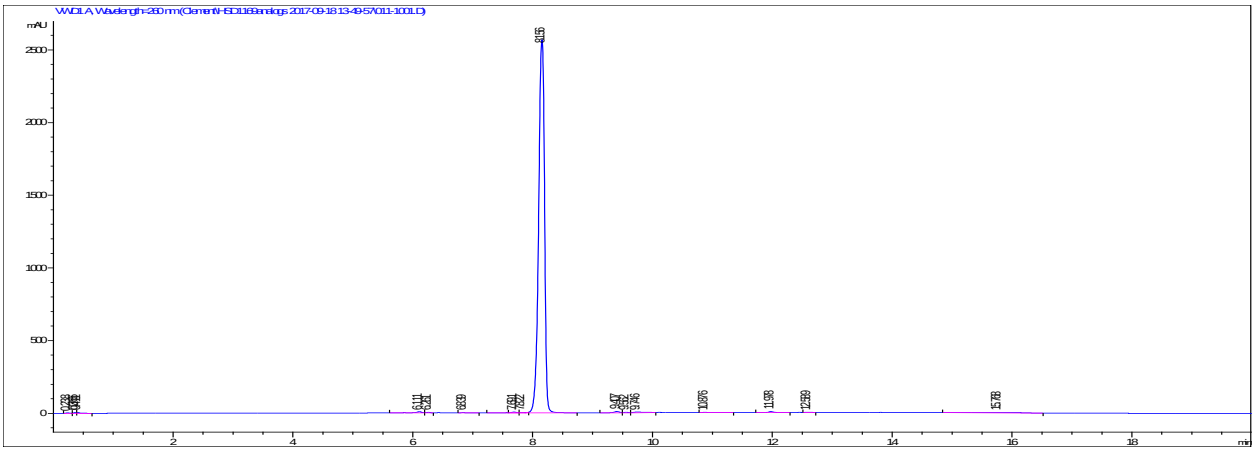
HPLC of compound 7



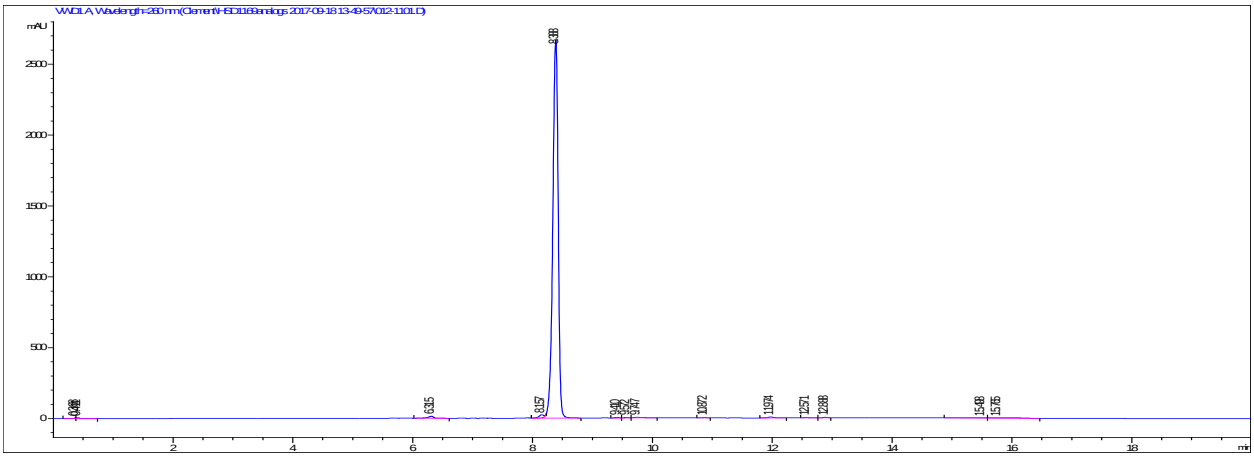
HPLC of compound 8



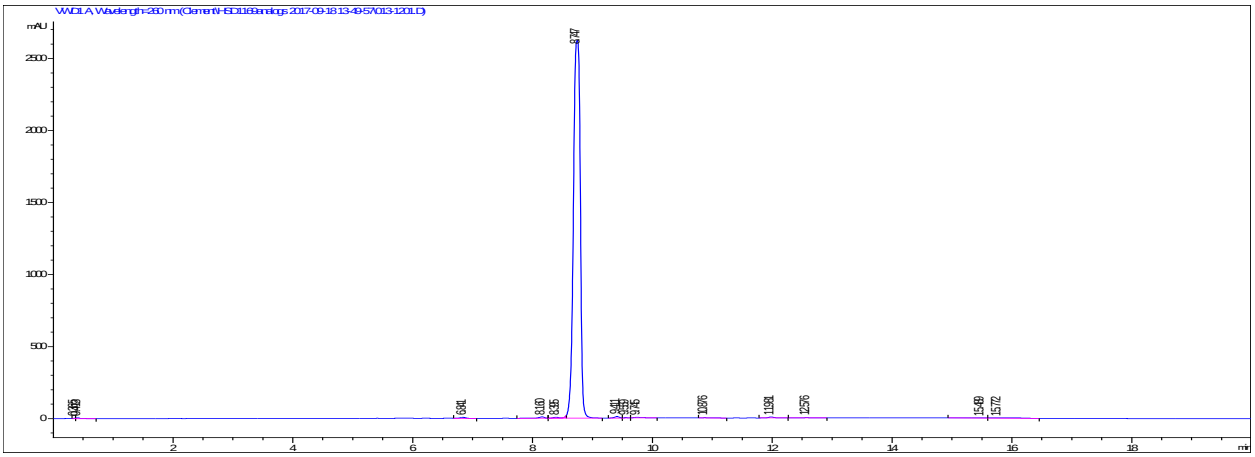
HPLC of compound 9



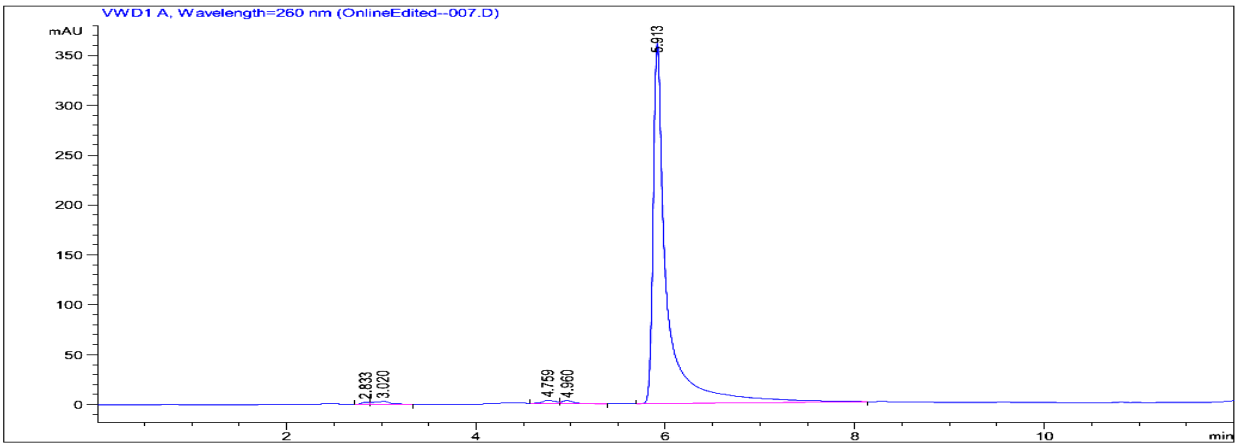
HPLC of compound 10



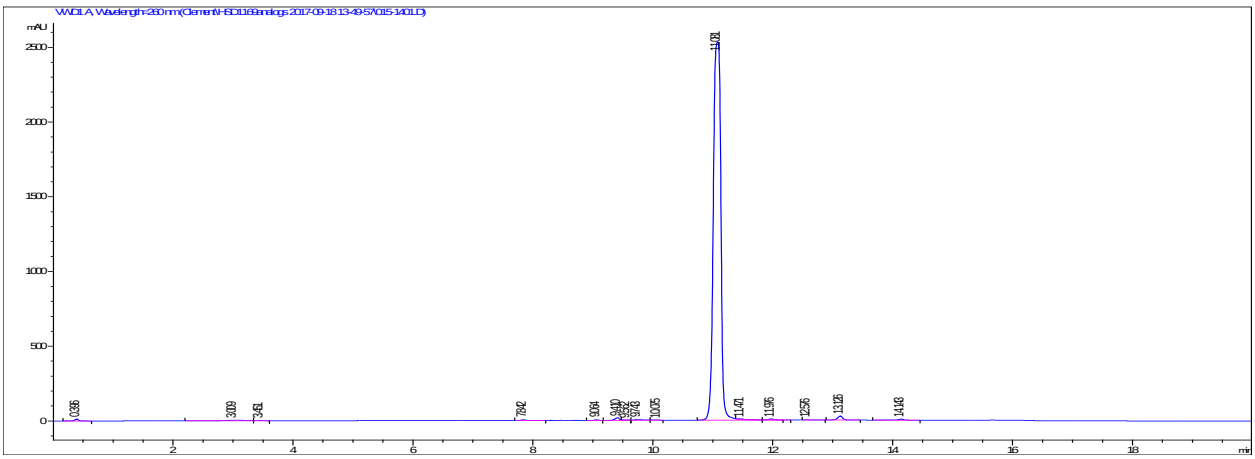
HPLC of compound 11



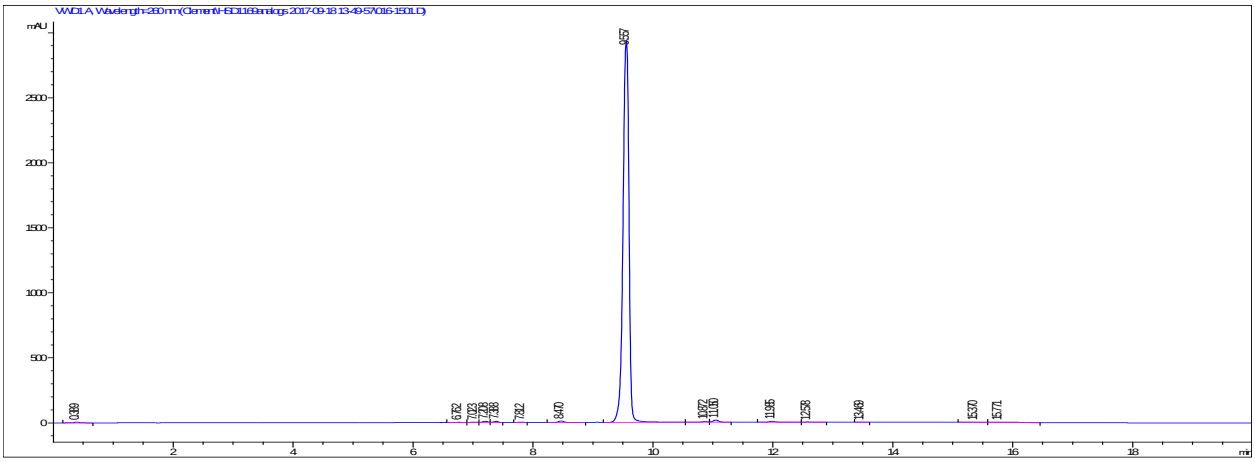
HPLC of compound 12



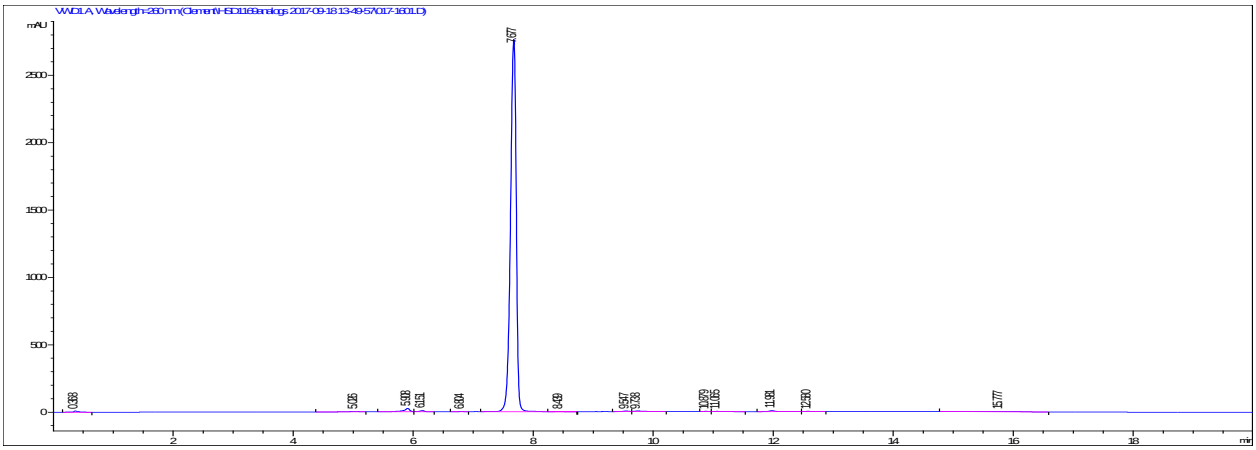
HPLC of compound 13



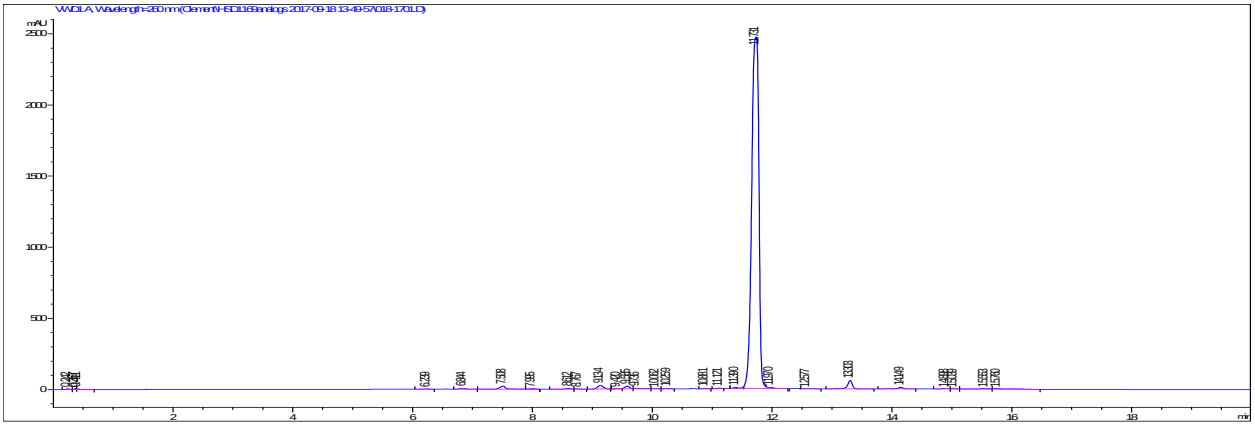
HPLC of compound 14



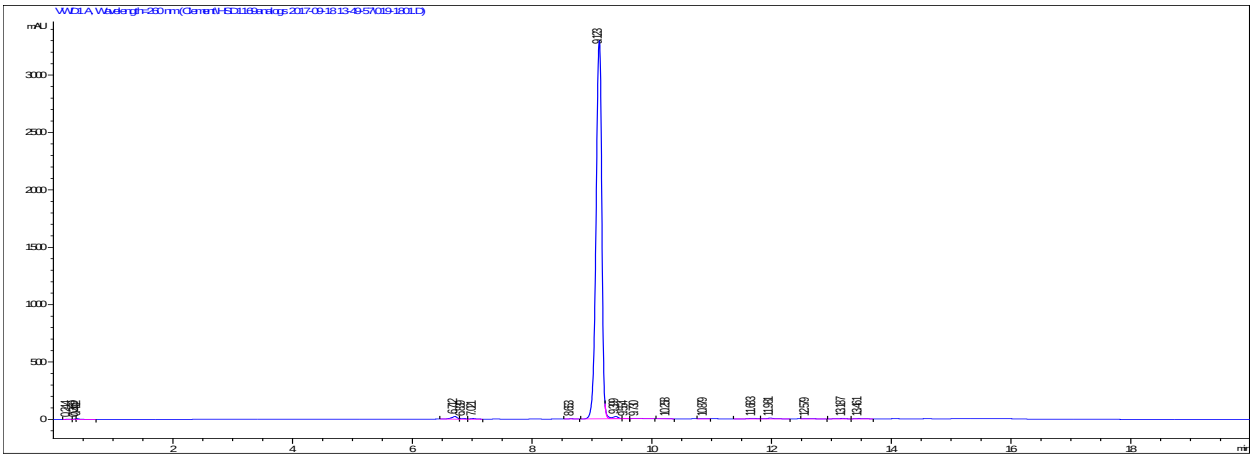
HPLC of compound 15



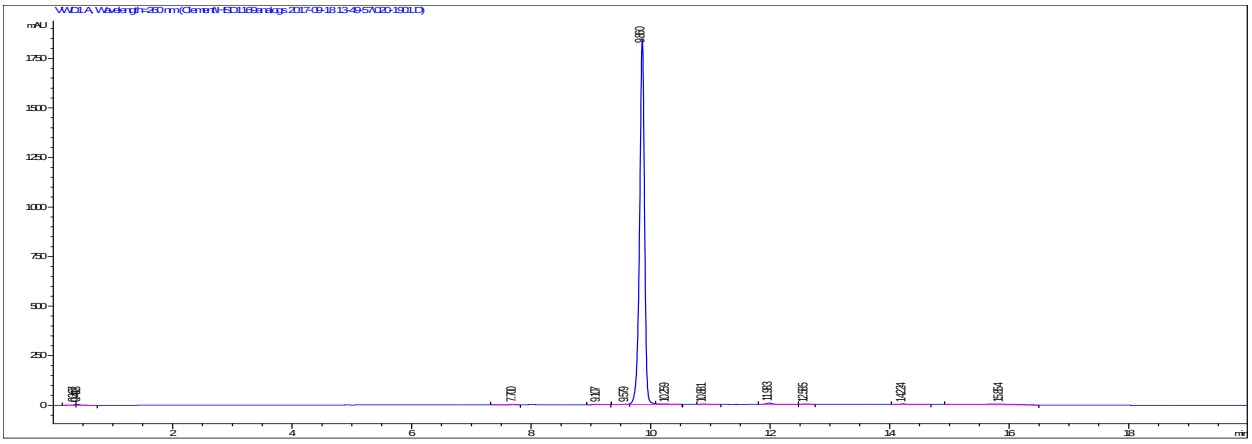
HPLC of compound 16



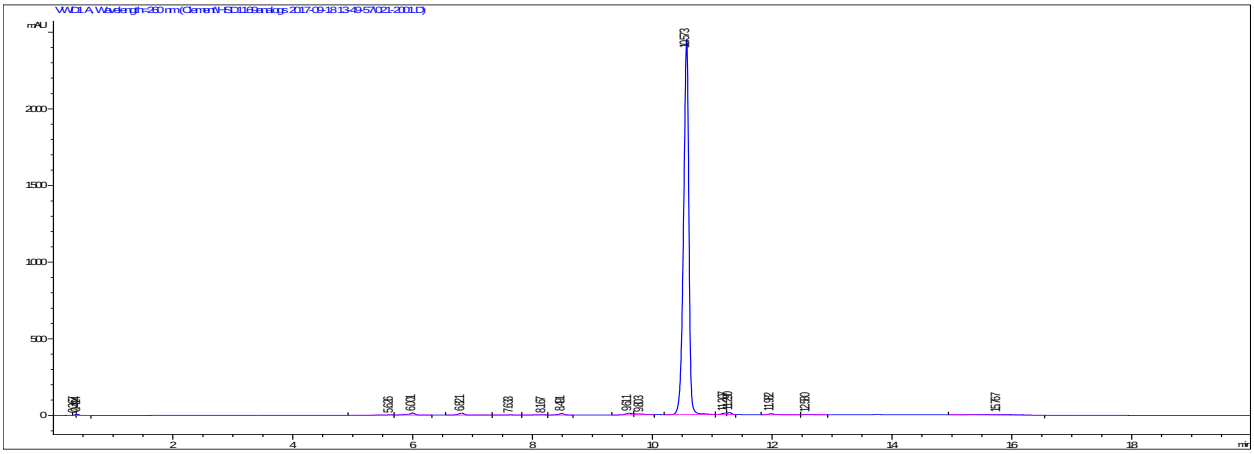
HPLC of compound 17



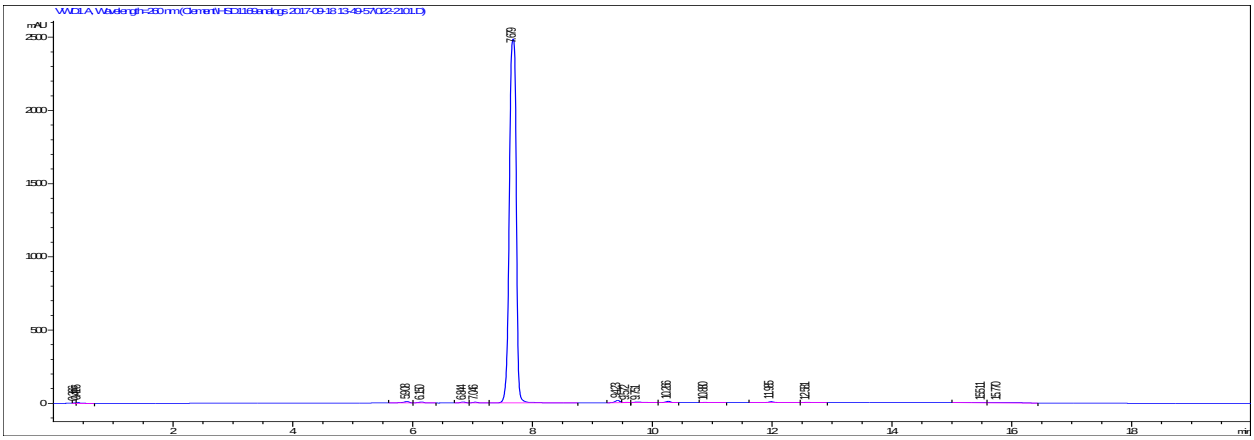
HPLC of compound 18



HPLC of compound 19

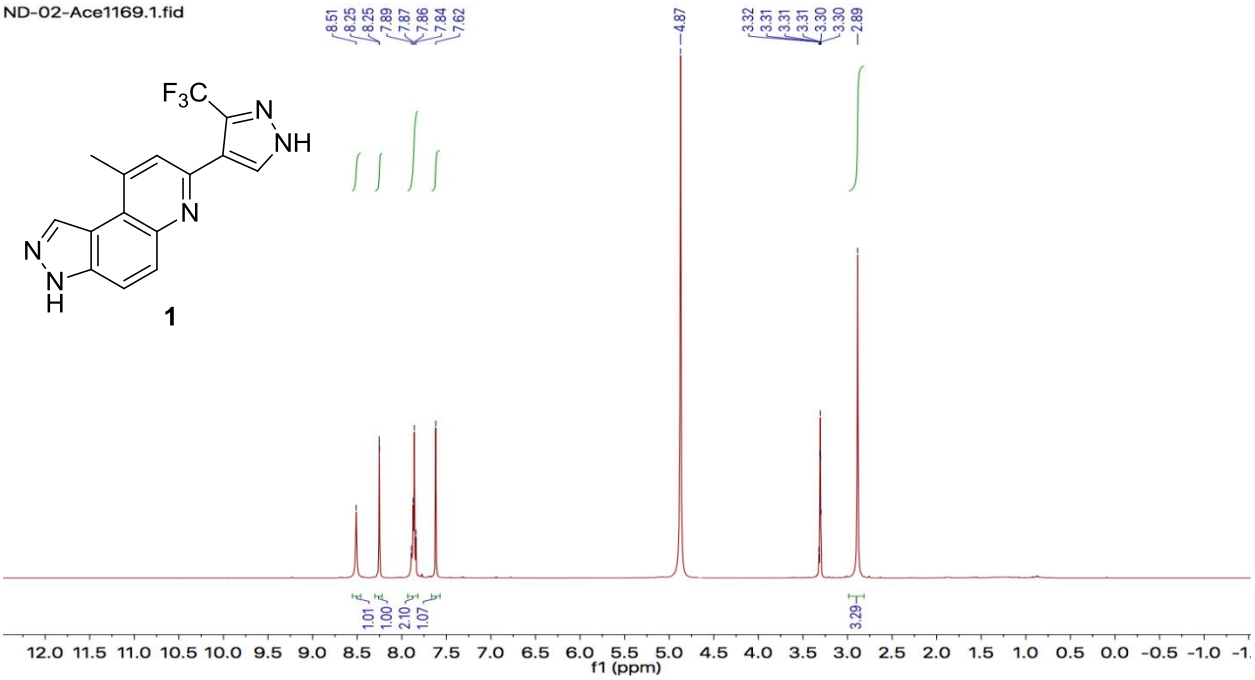


HPLC of compound 20

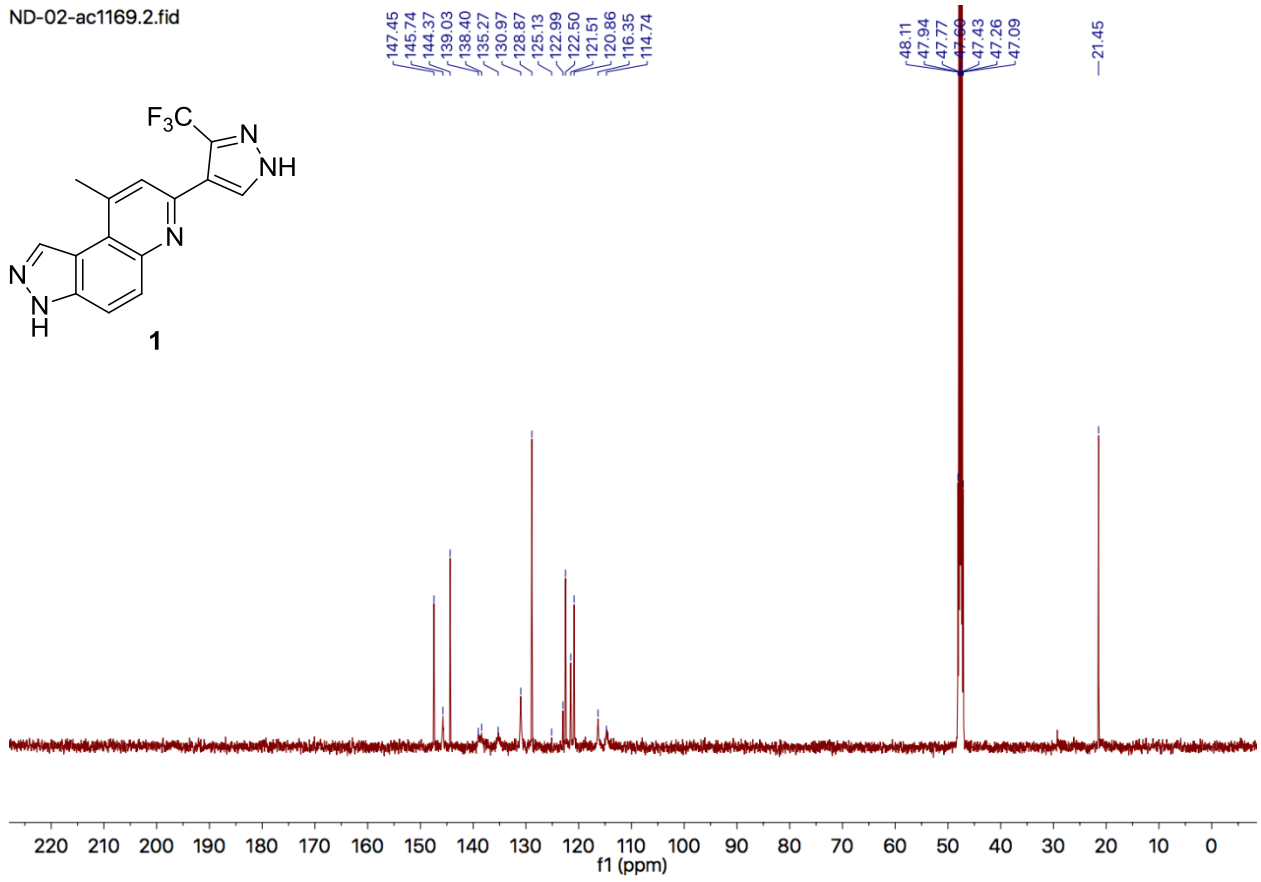


HPLC of compound 21

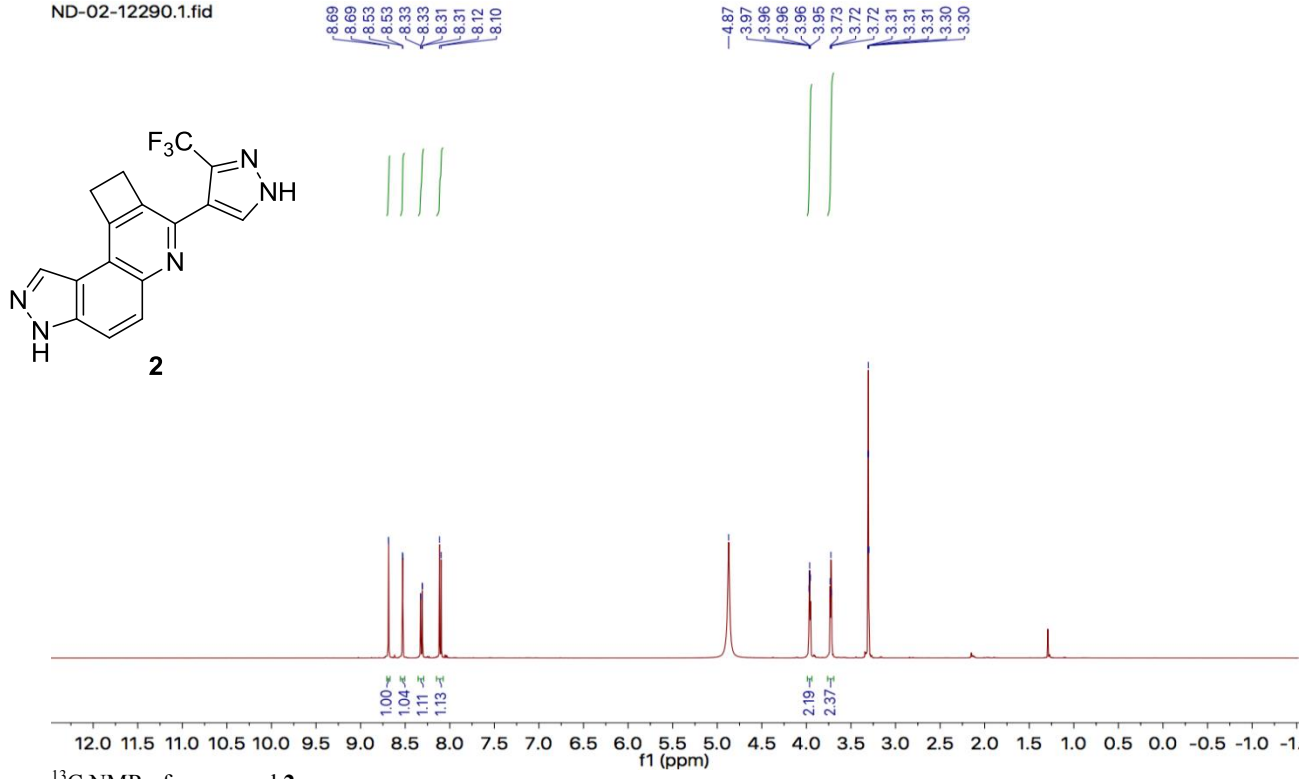
^{1}H NMR of compound **1**



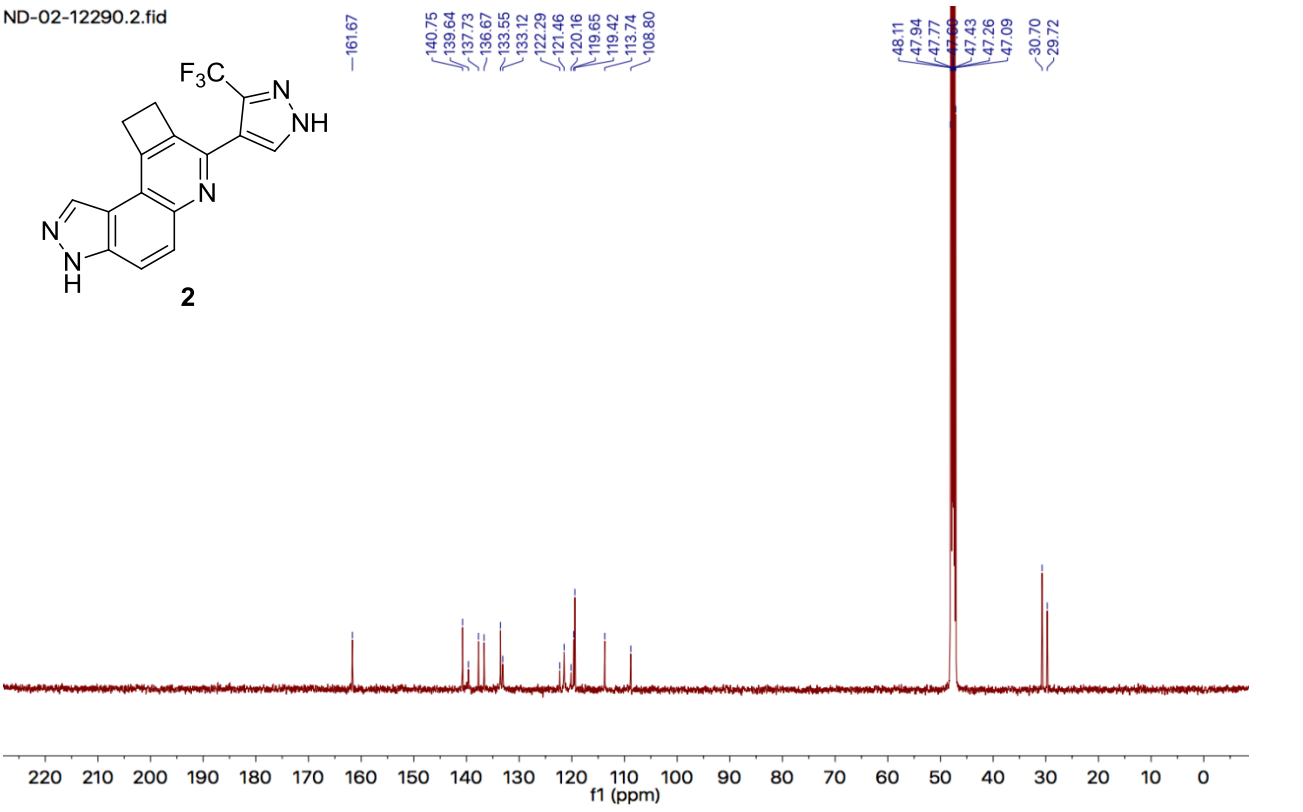
^{13}C NMR of compound **1**



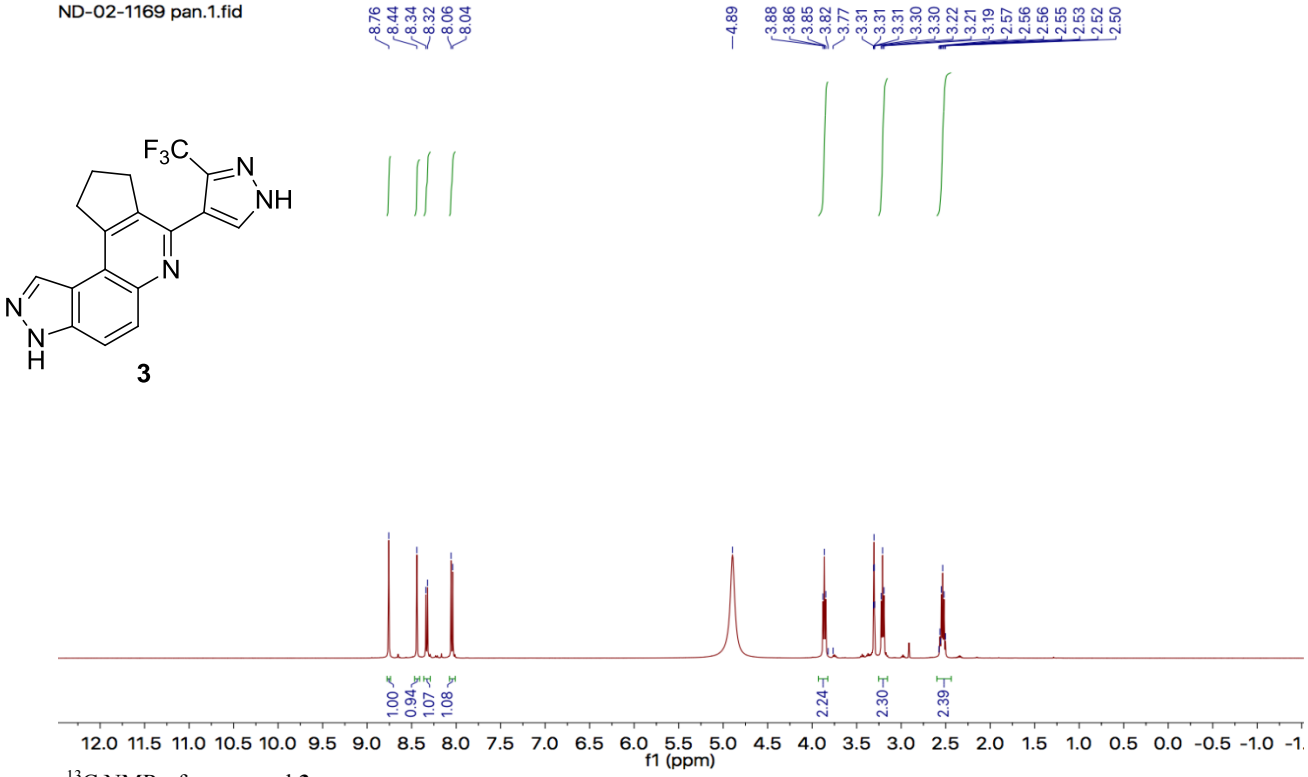
^{1}H NMR of compound **2**



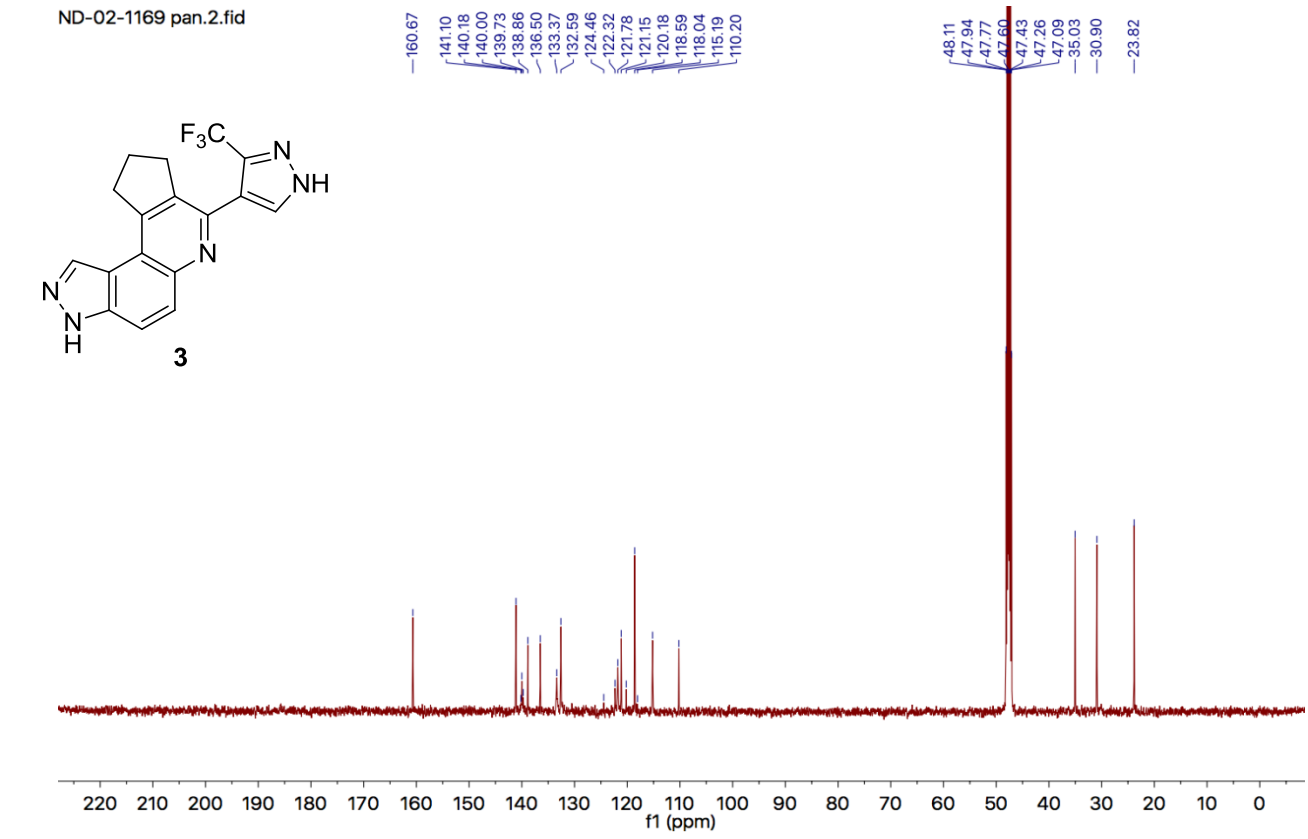
^{13}C NMR of compound **2**



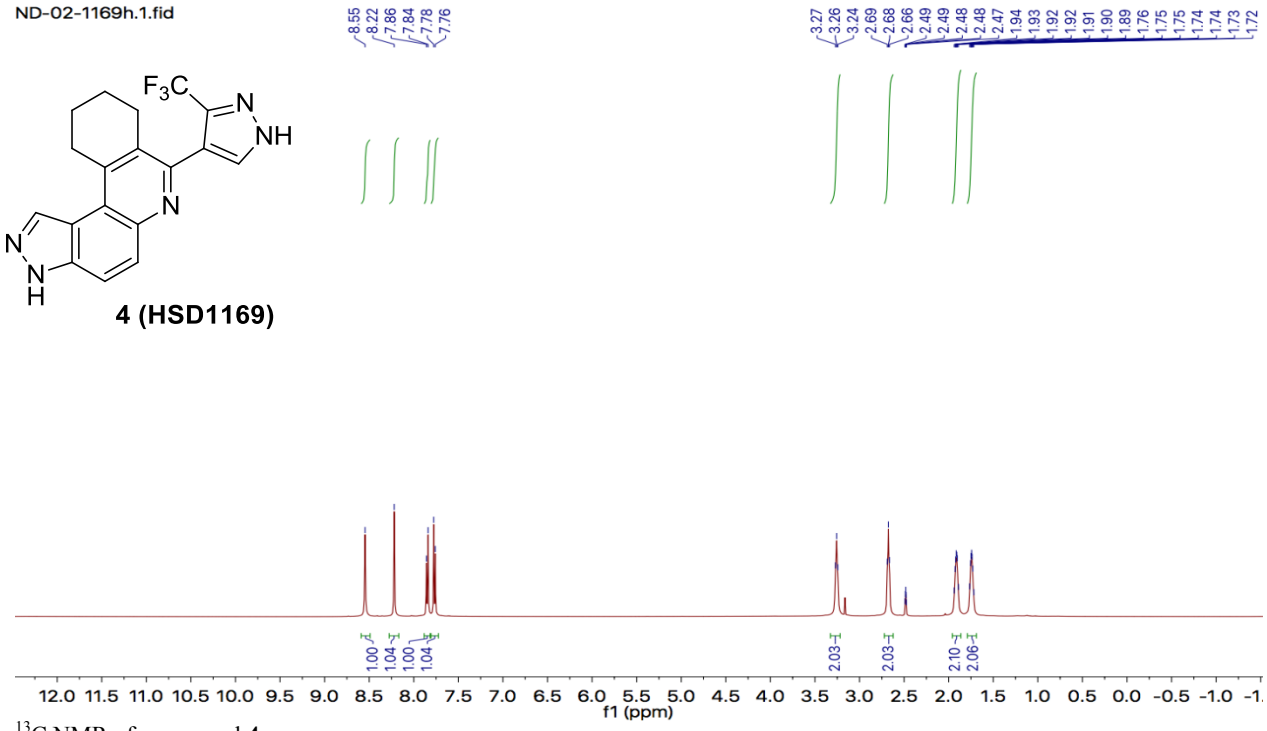
^{1}H NMR of compound **3**



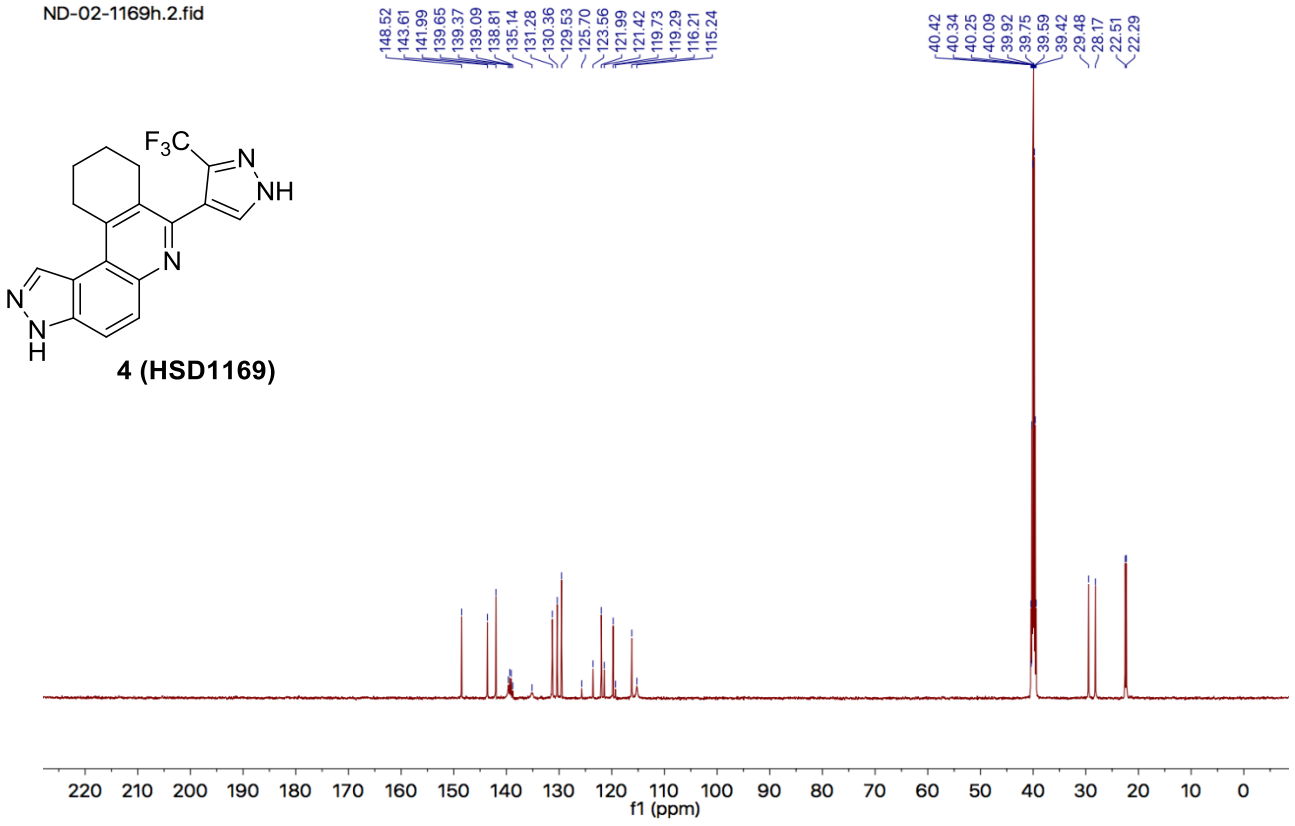
^{13}C NMR of compound **3**



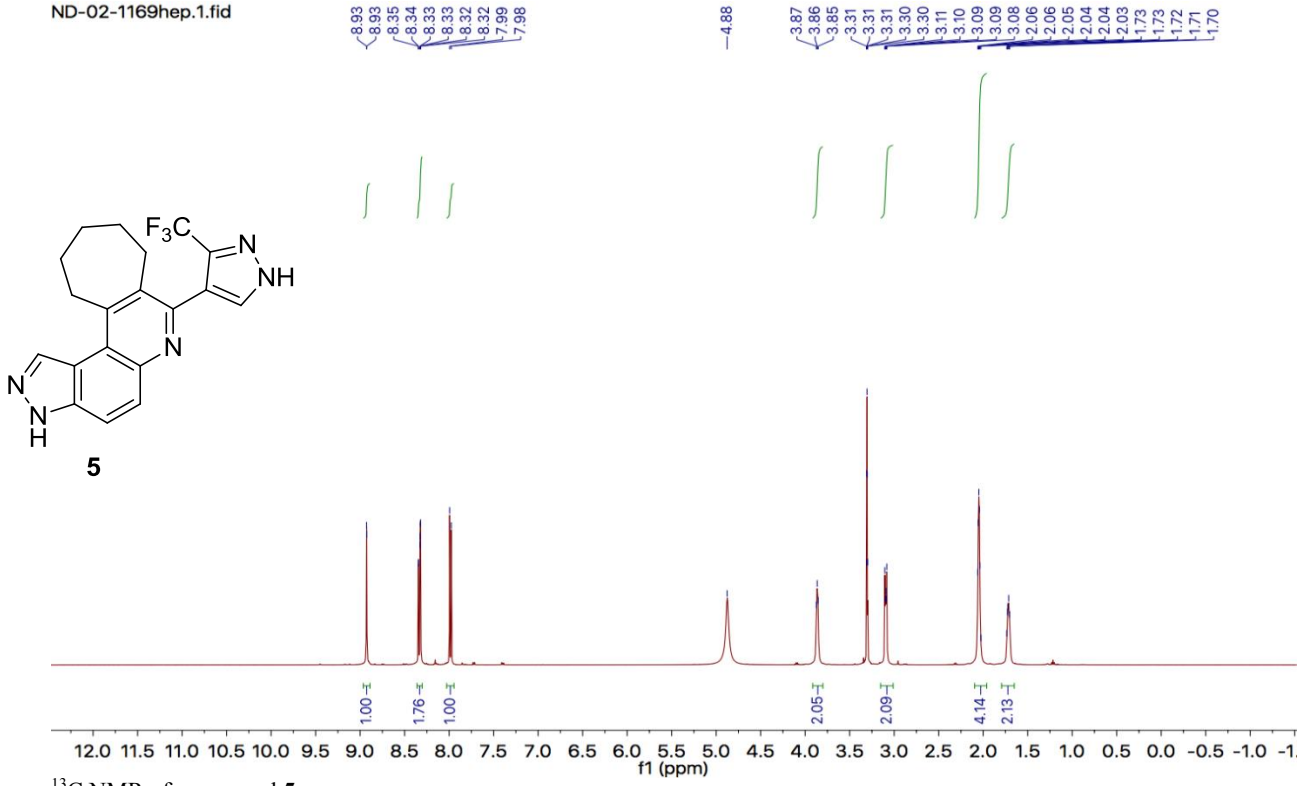
^{1}H NMR of compound **4**



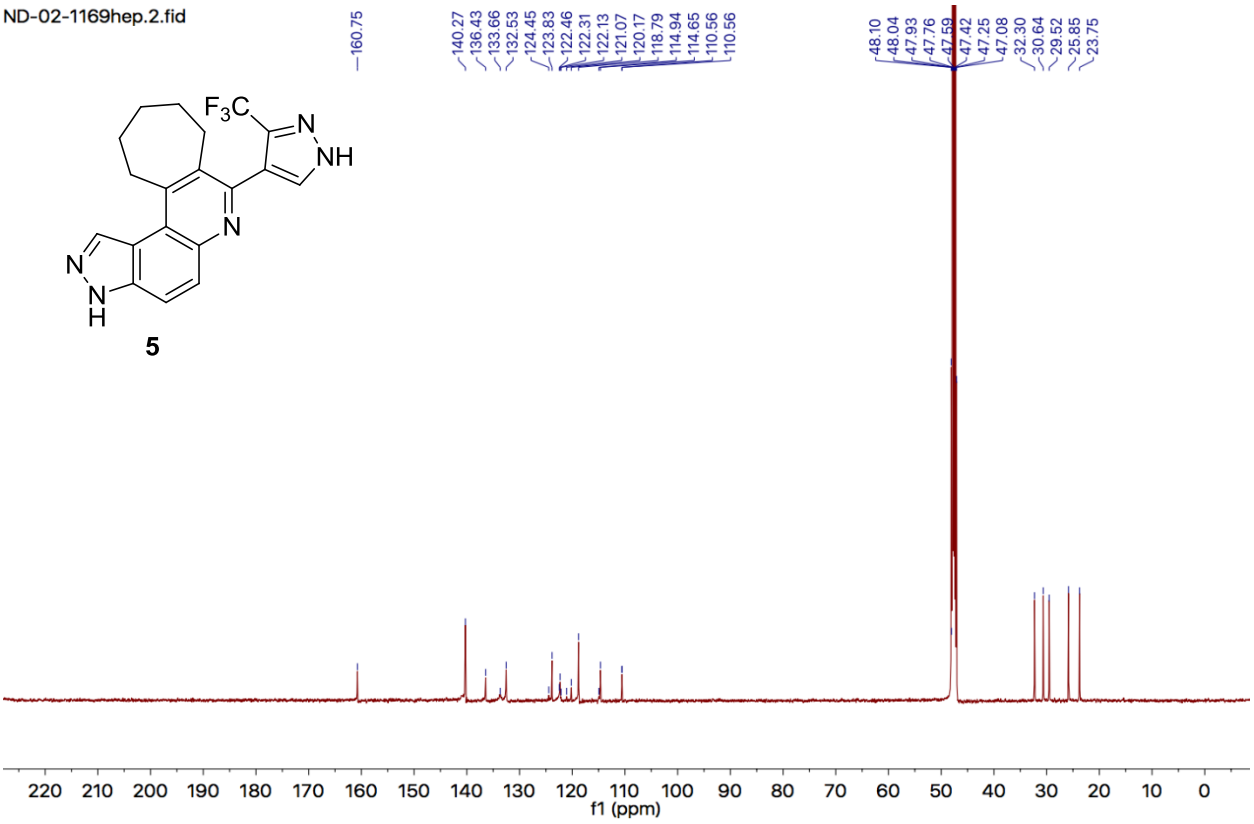
^{13}C NMR of compound **4**



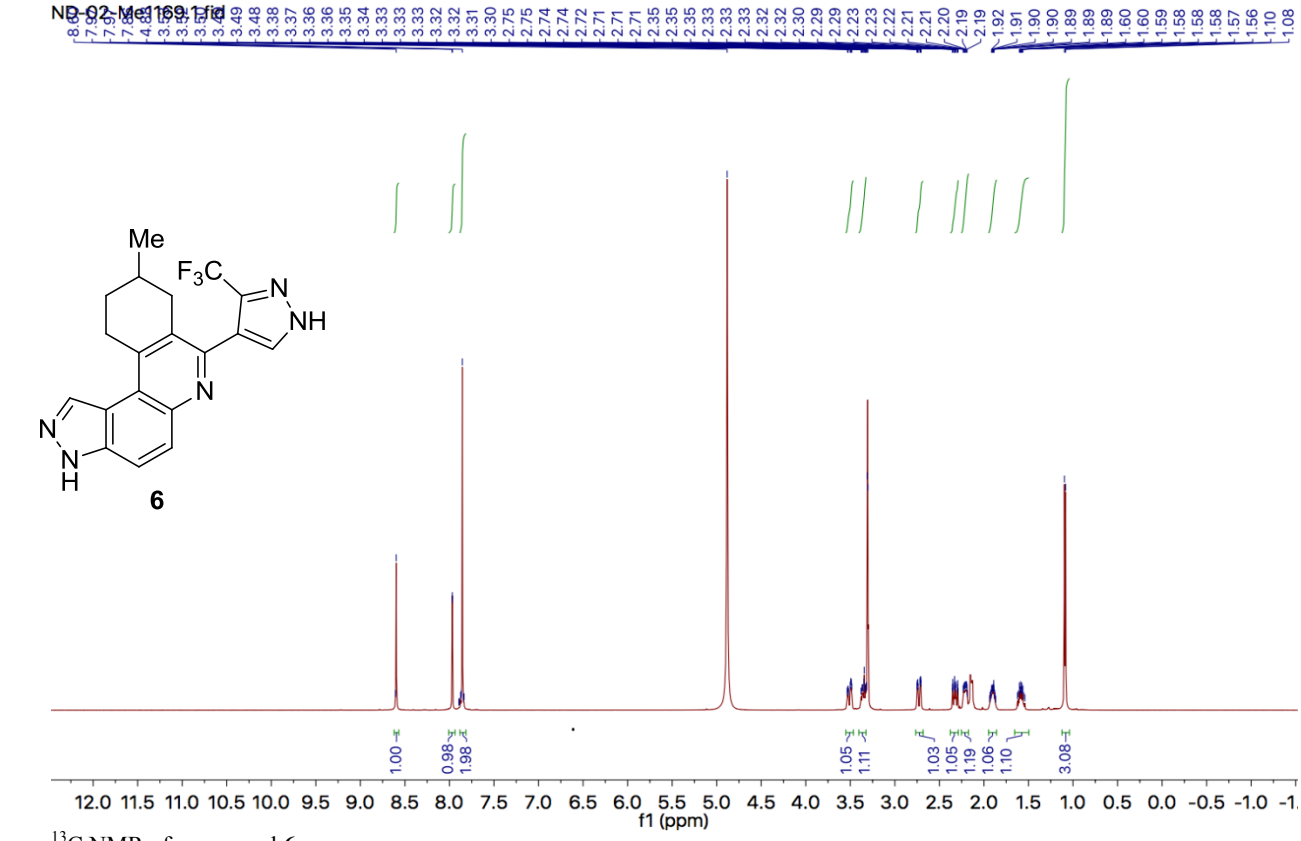
^{1}H NMR of compound **5**



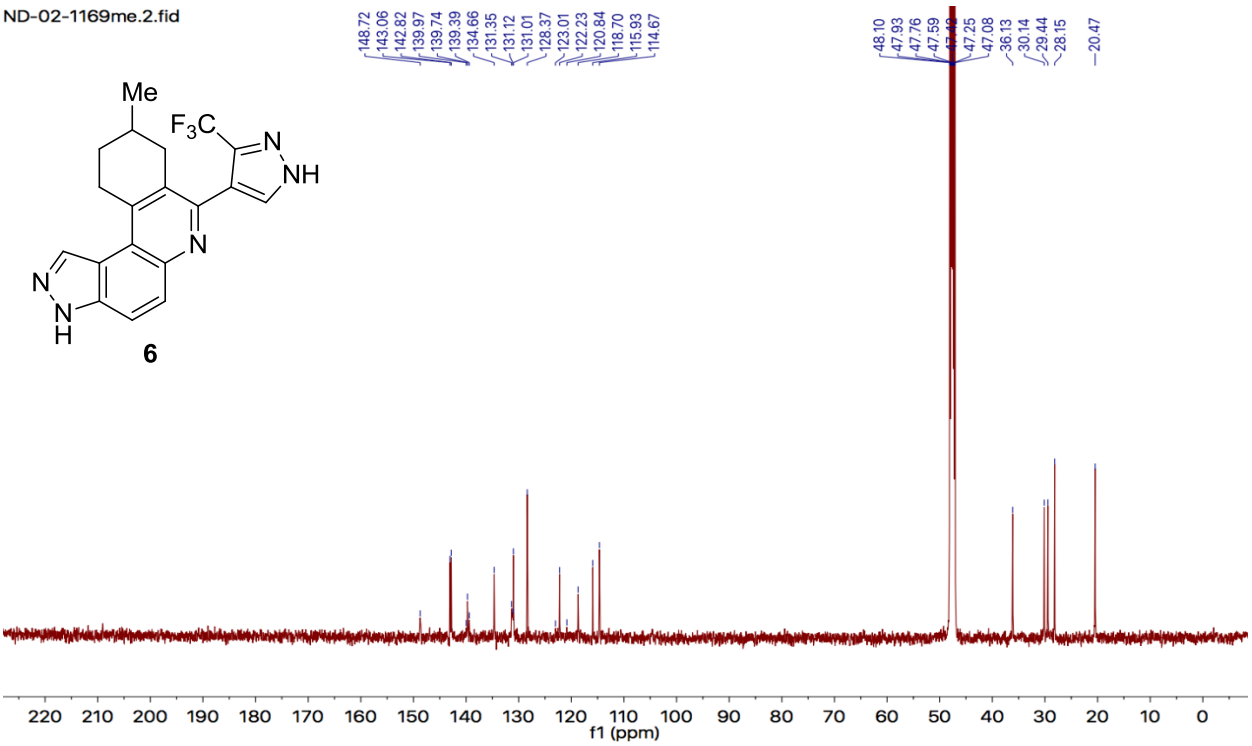
^{13}C NMR of compound **5**



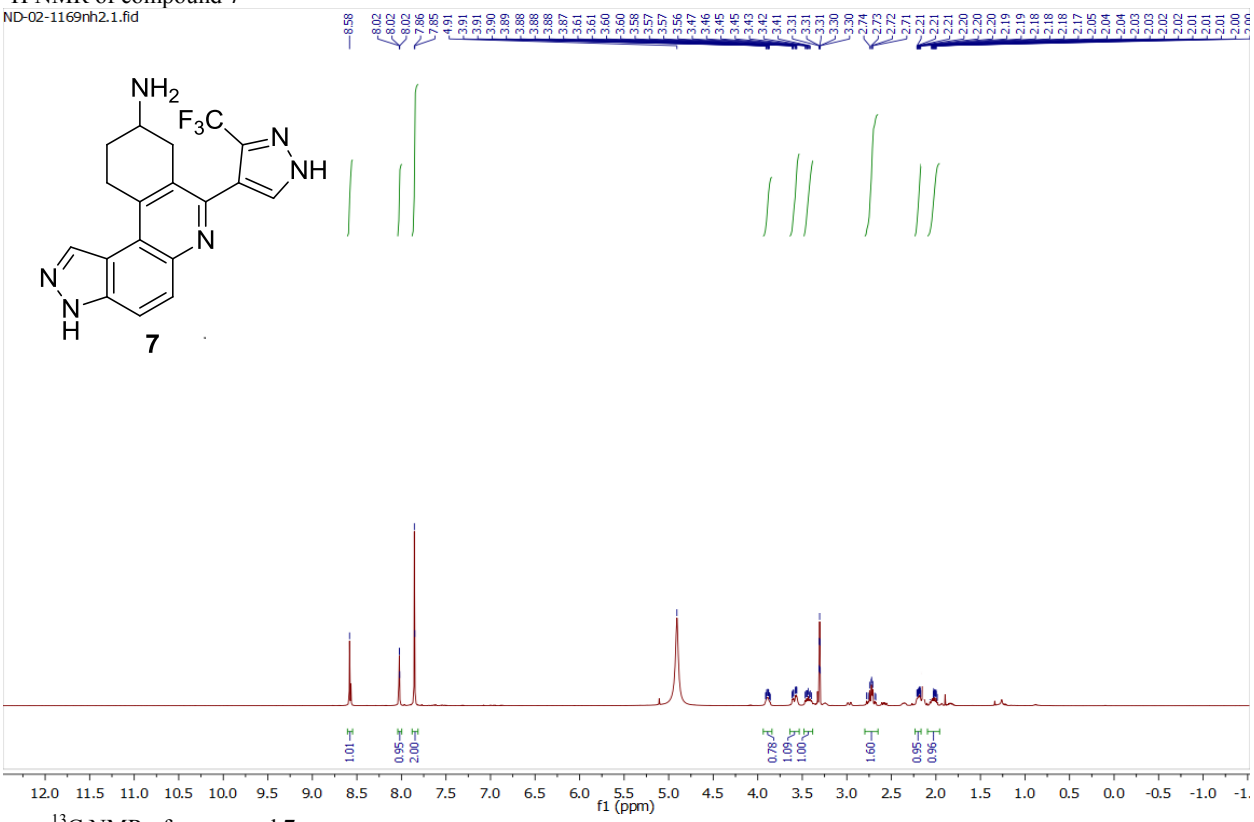
^{1}H NMR of compound **6**



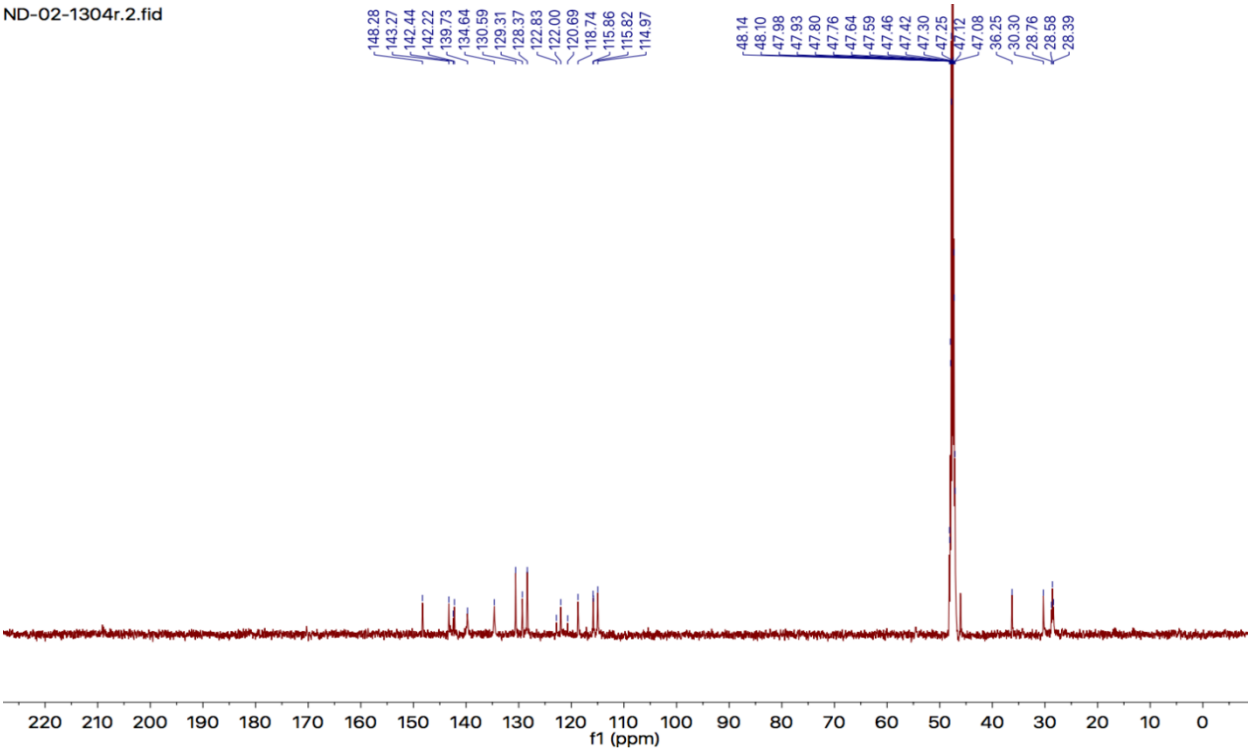
^{13}C NMR of compound **6**



^{1}H NMR of compound **7**

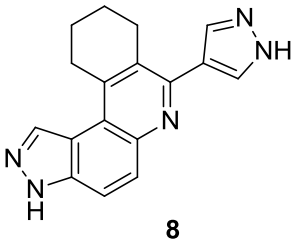


^{13}C NMR of compound **7**



^{1}H NMR of compound **8**

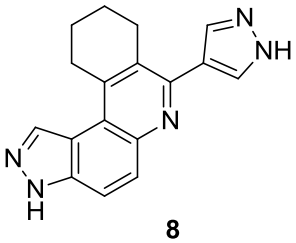
ND-02-1077r.1.fid



8

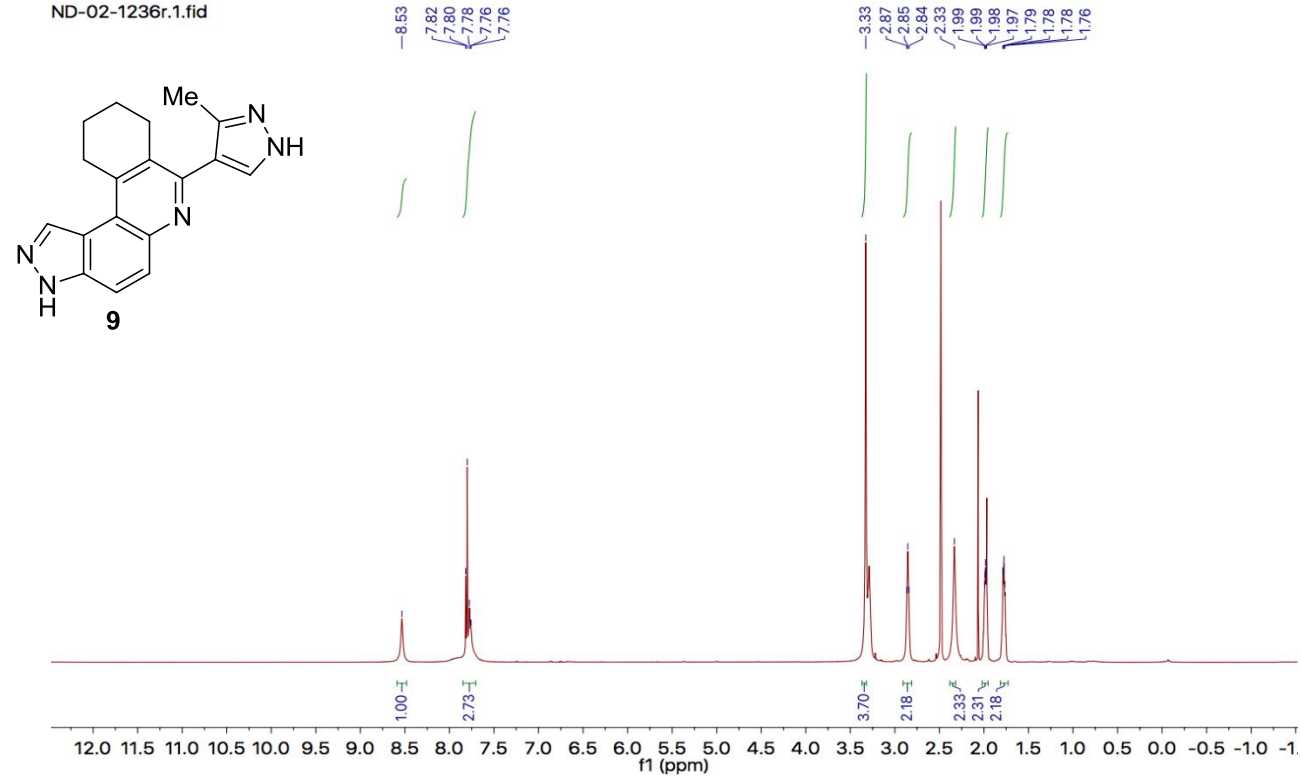
^{13}C NMR of compound **8**

ND-02-1077r.2.fid

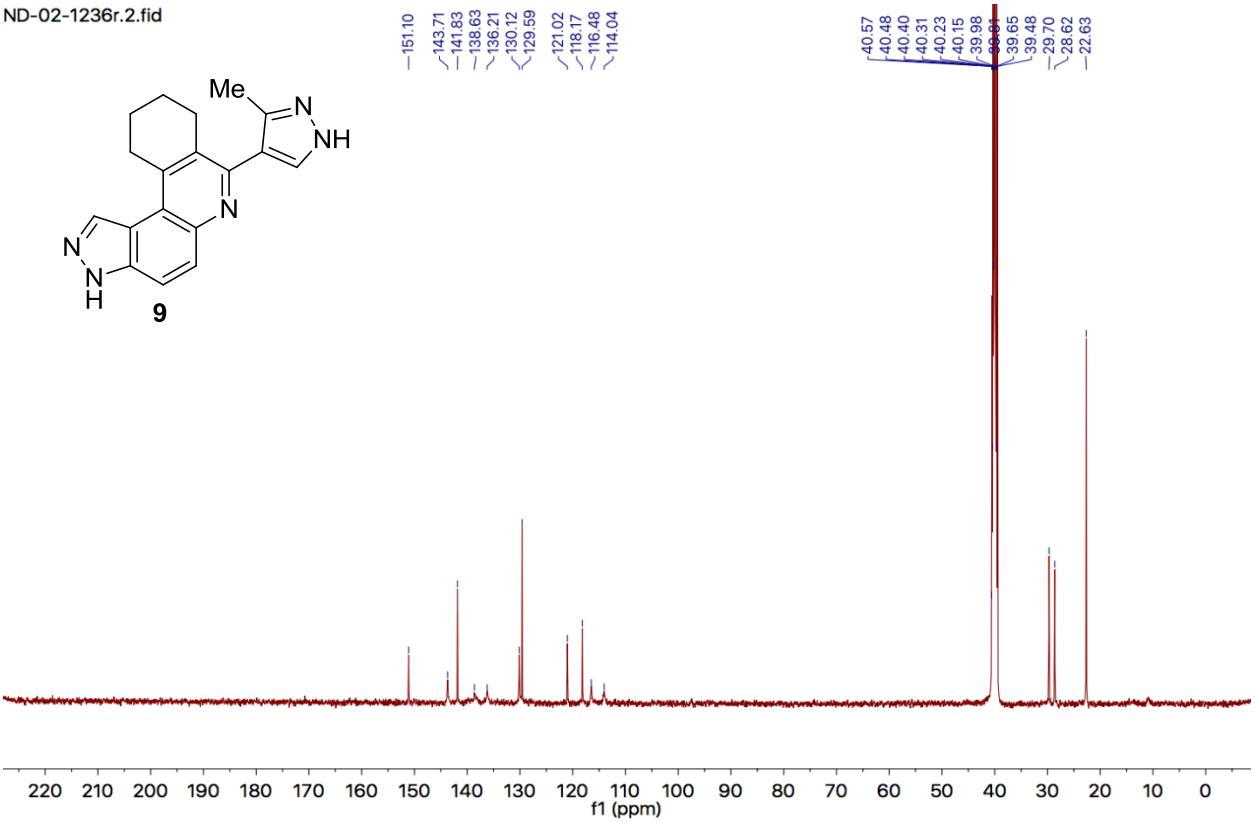


8

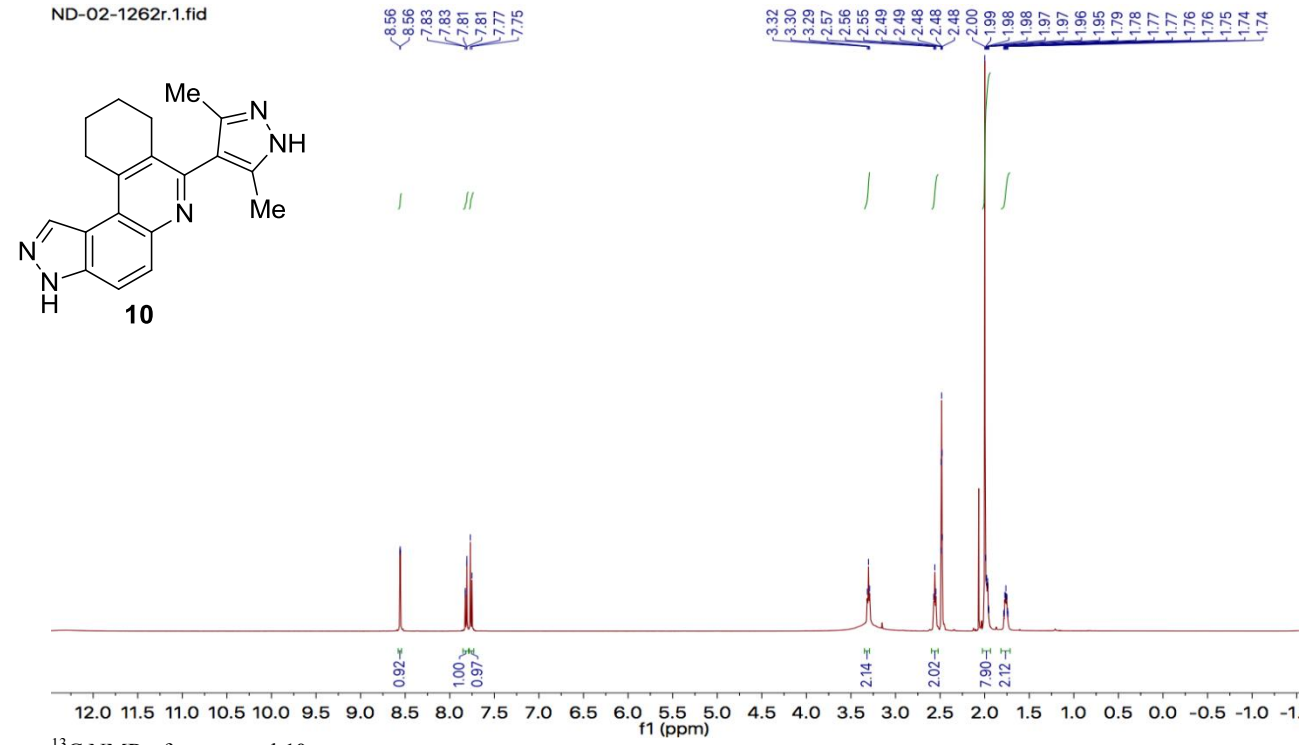
^{1}H NMR of compound **9**



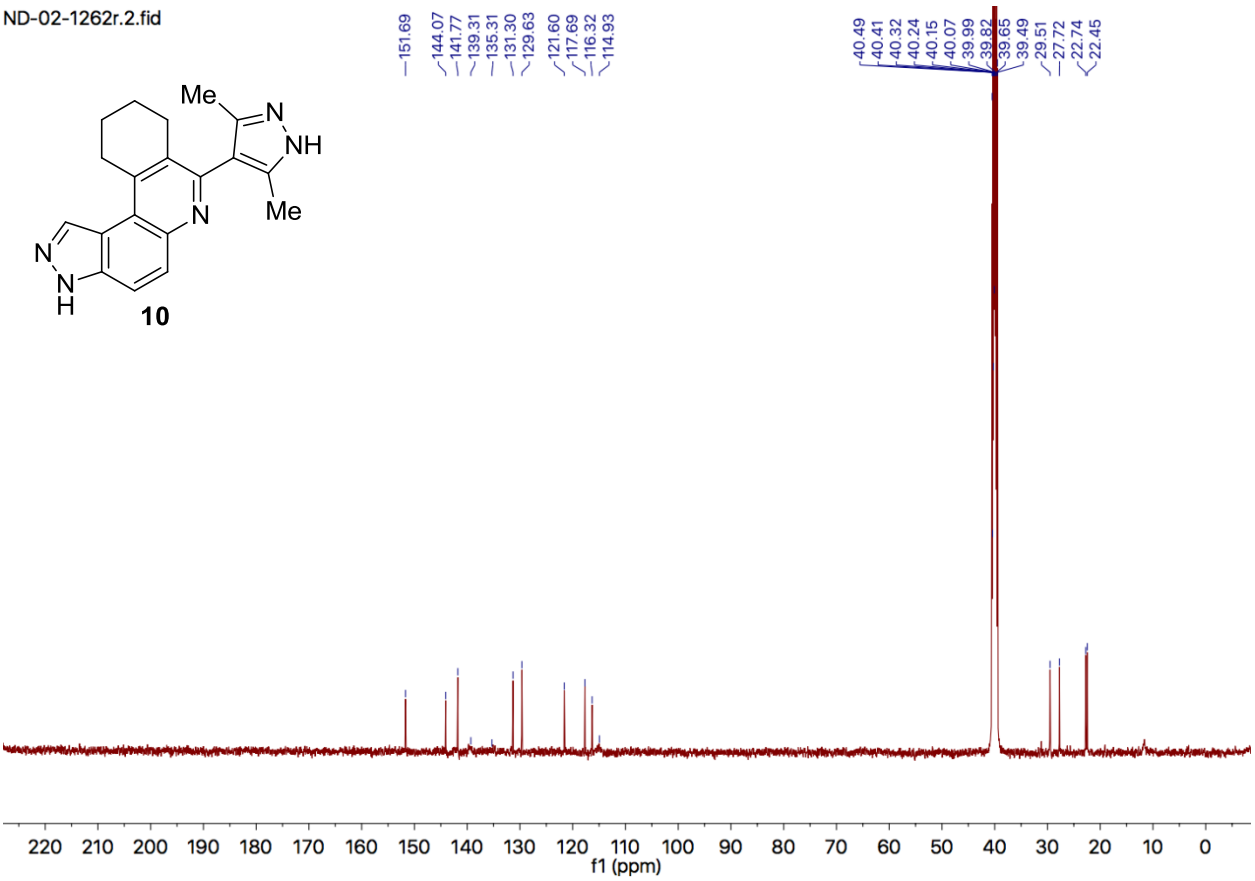
^{13}C NMR of compound **9**



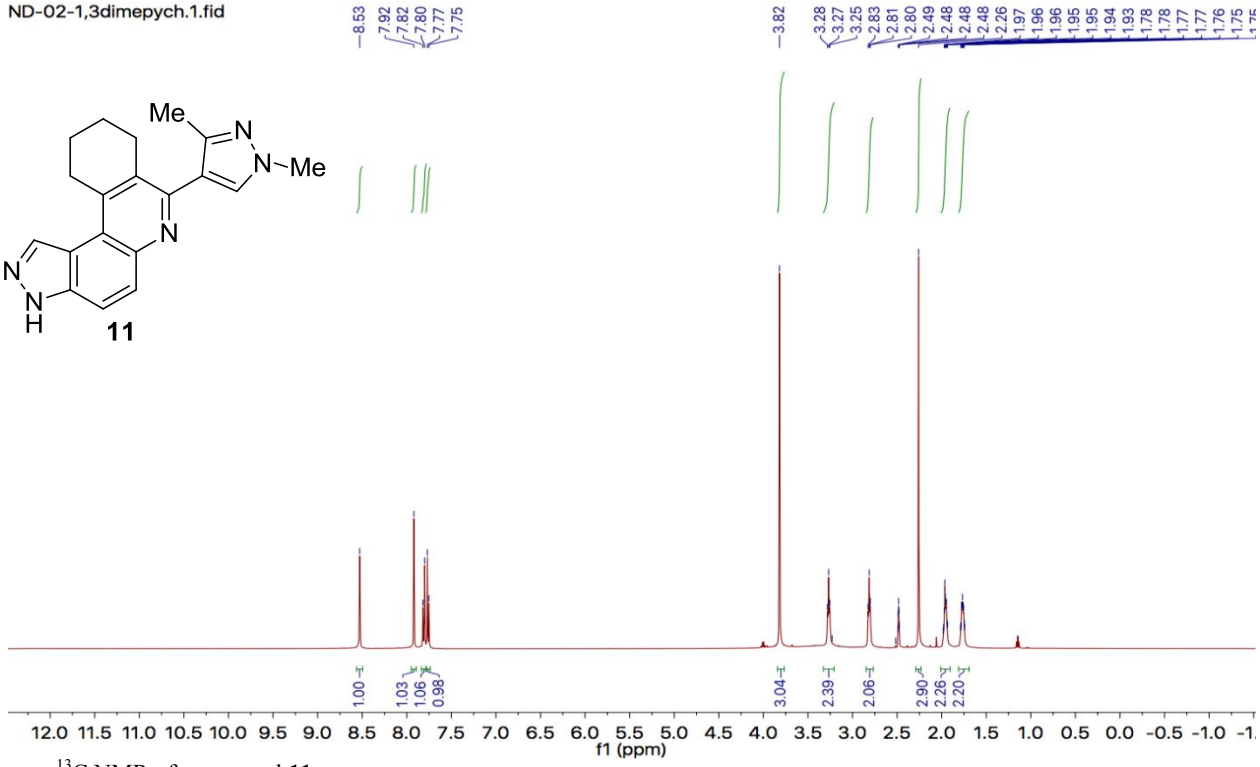
^{1}H NMR of compound **10**



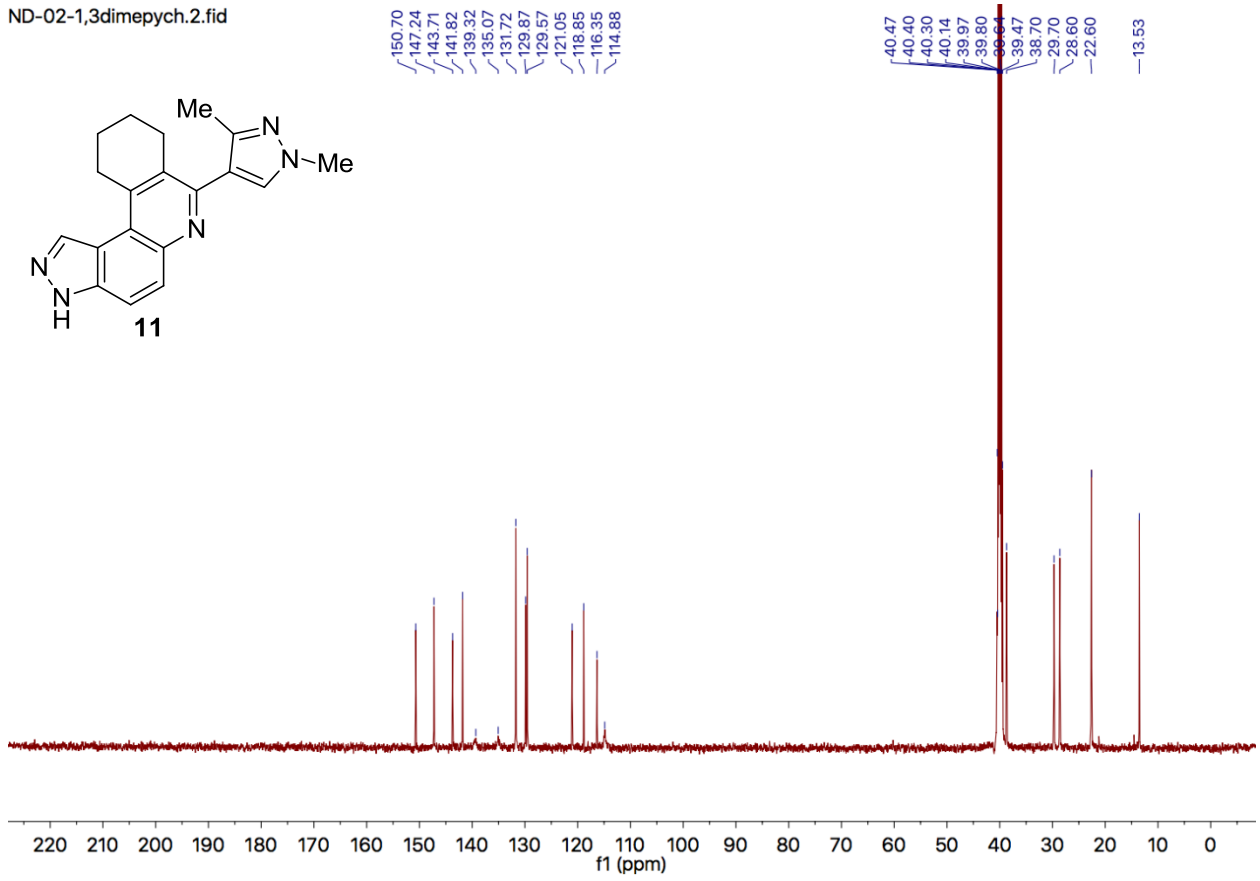
^{13}C NMR of compound **10**



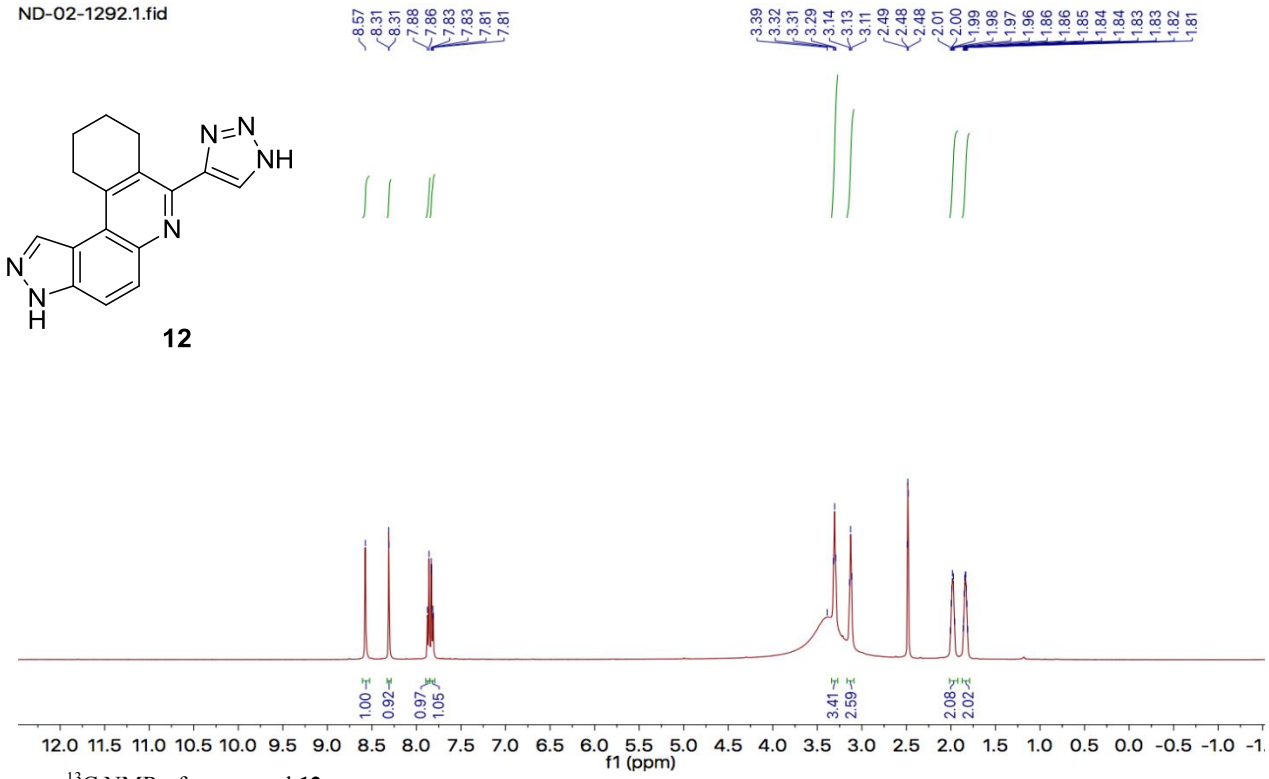
^{1}H NMR of compound **11**



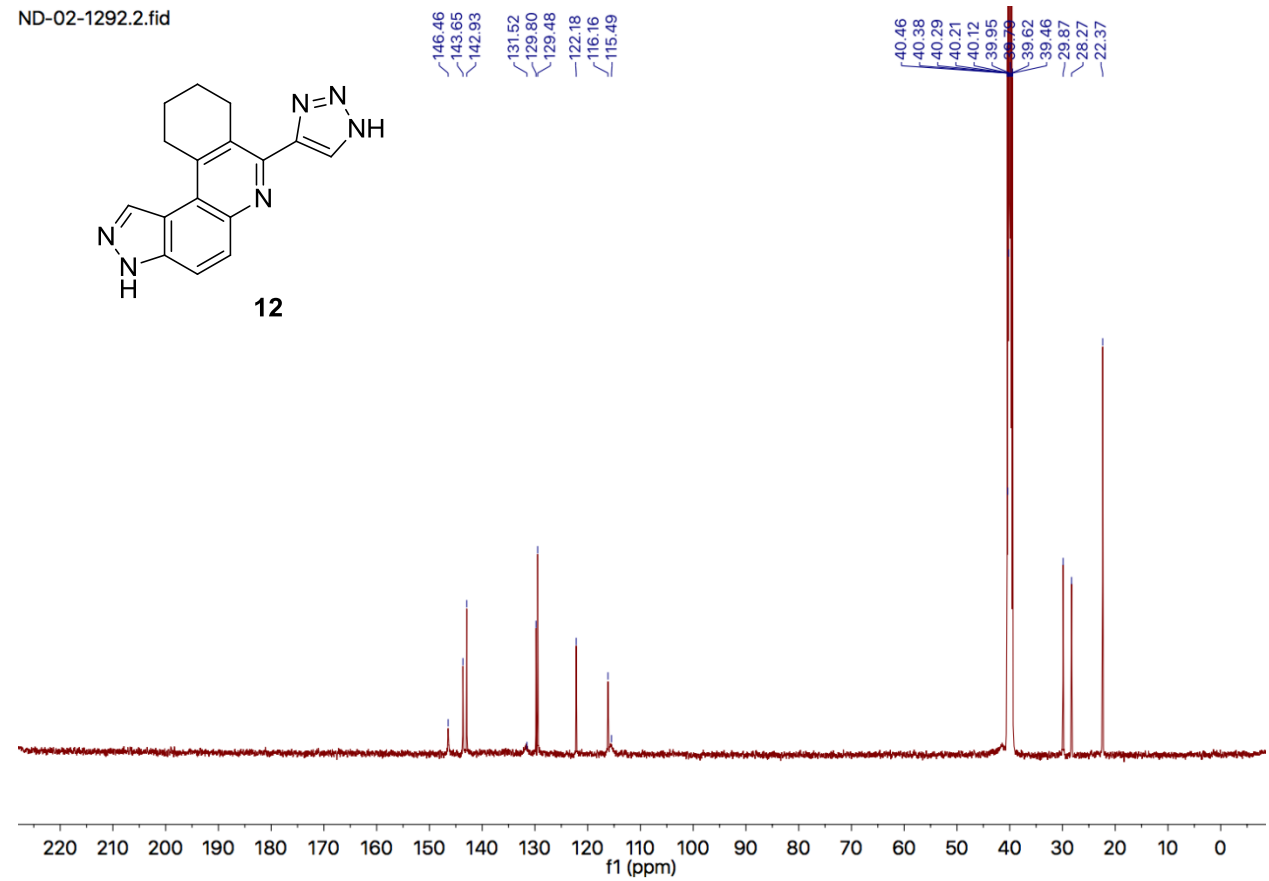
^{13}C NMR of compound **11**



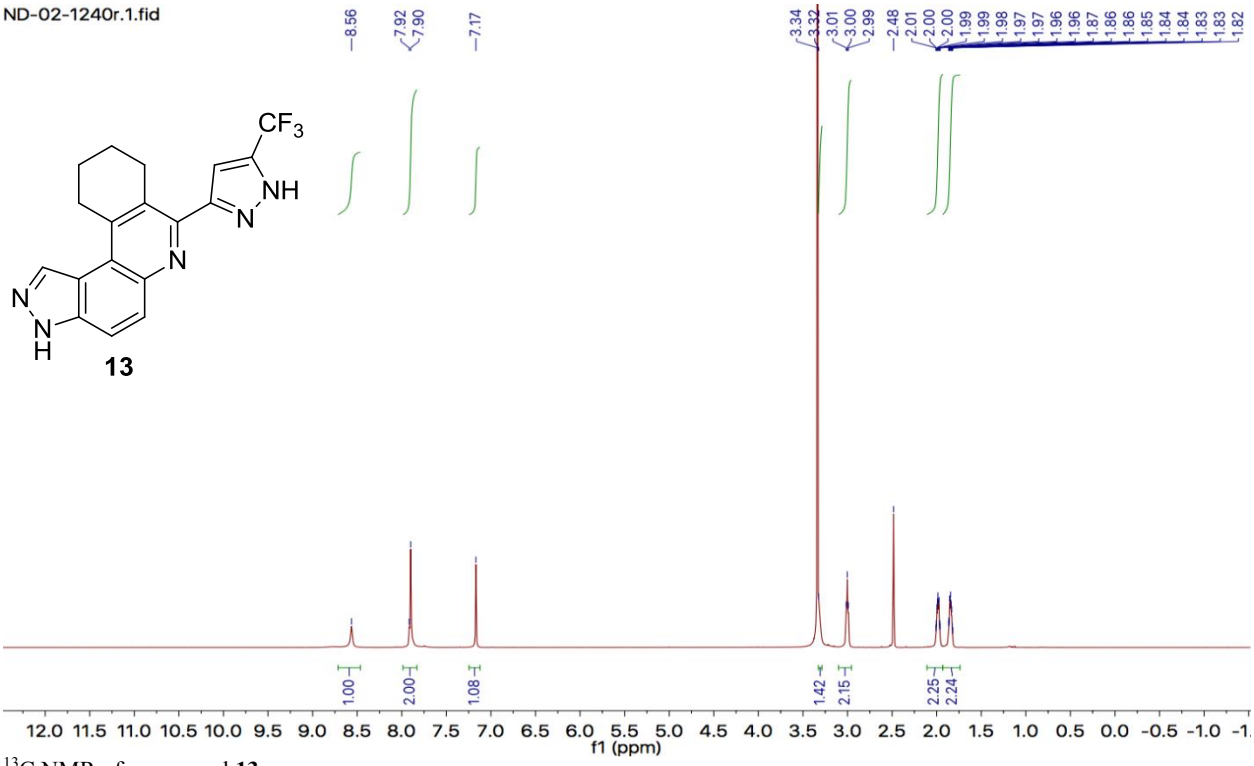
^{1}H NMR of compound **12**



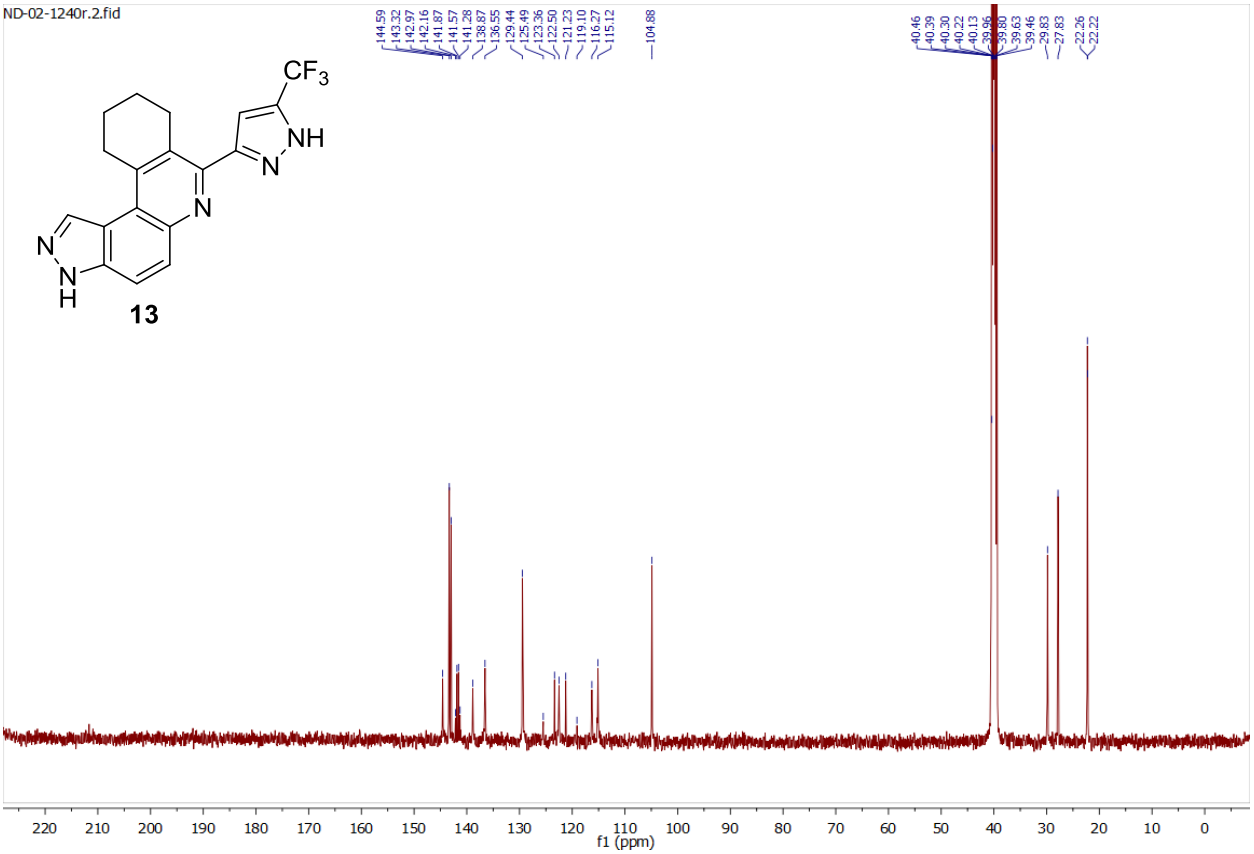
^{13}C NMR of compound **12**



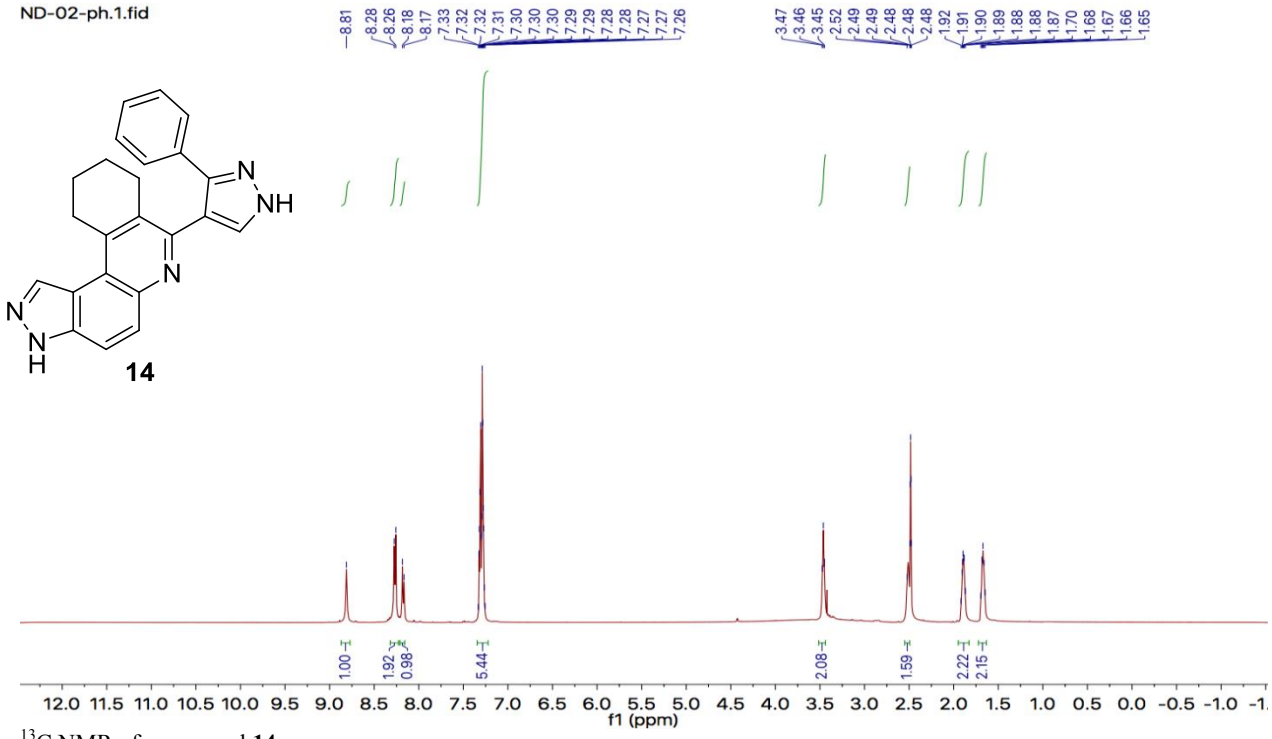
^{1}H NMR of compound **13**



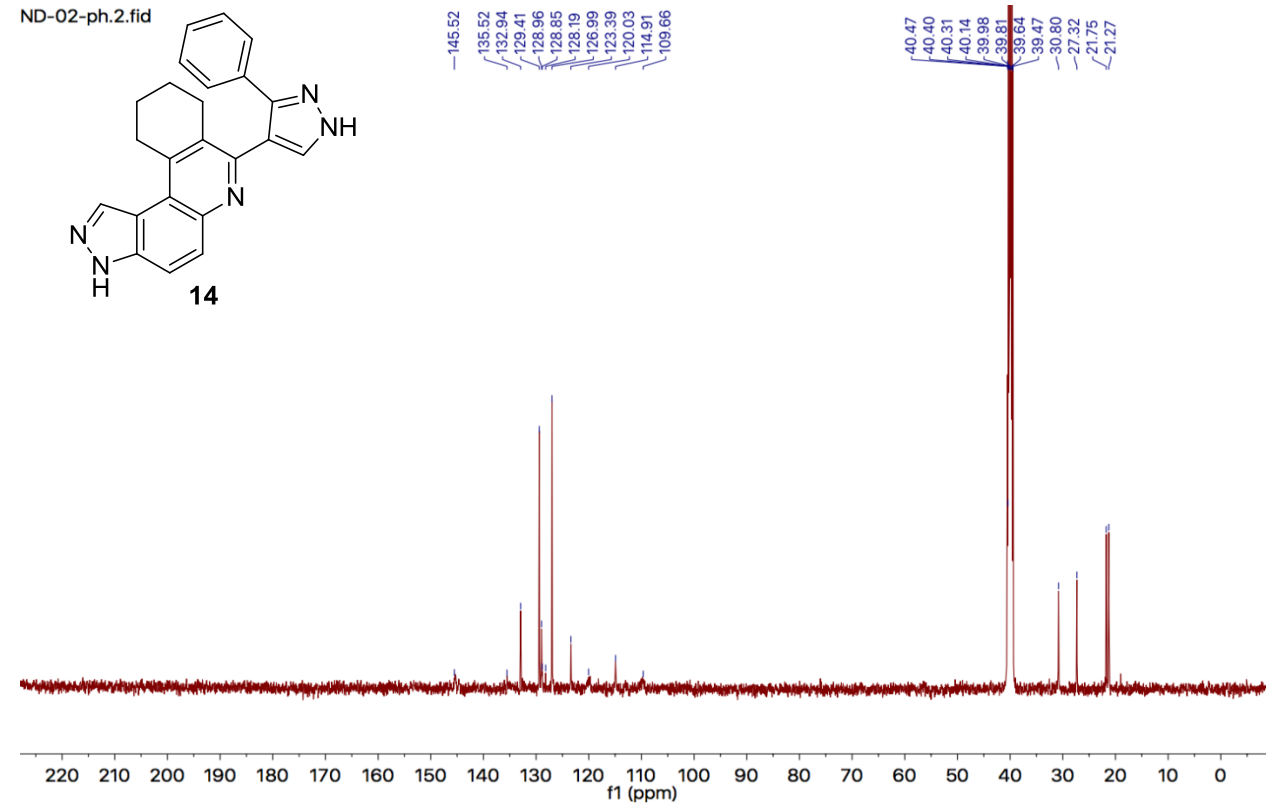
^{13}C NMR of compound **13**



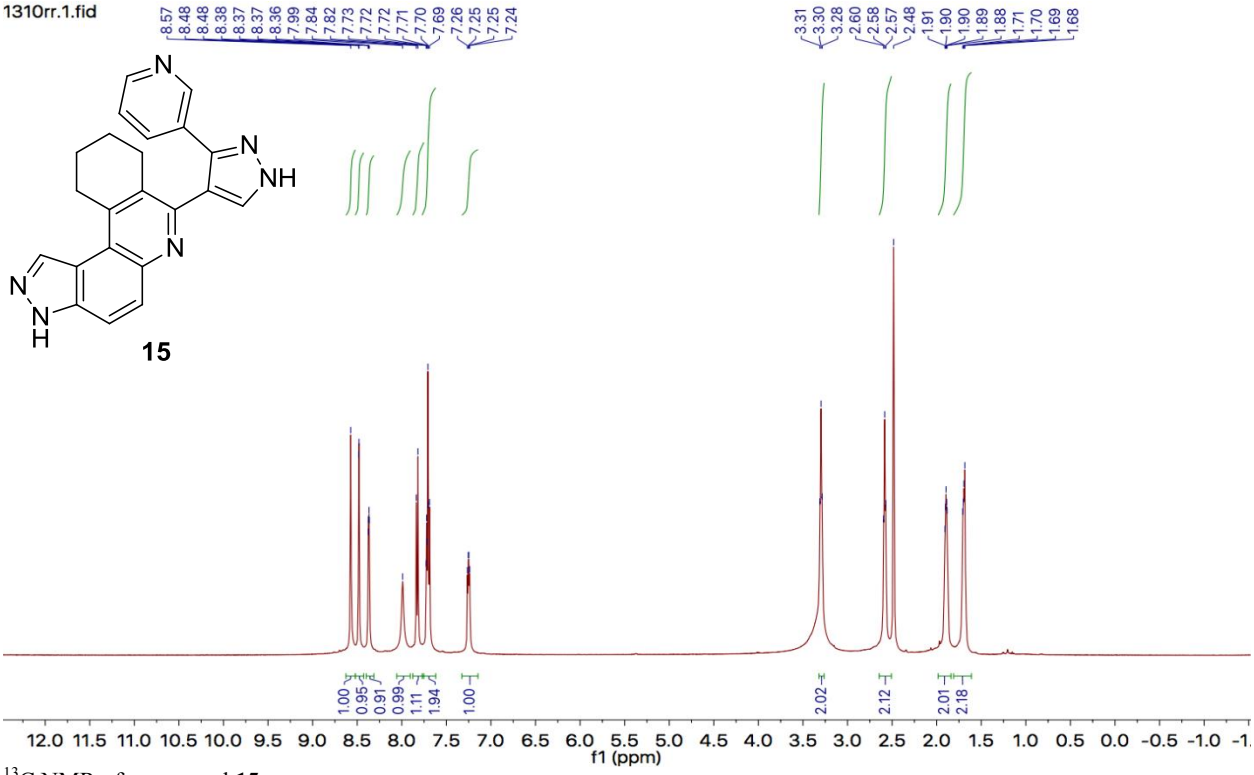
^{1}H NMR of compound **14**



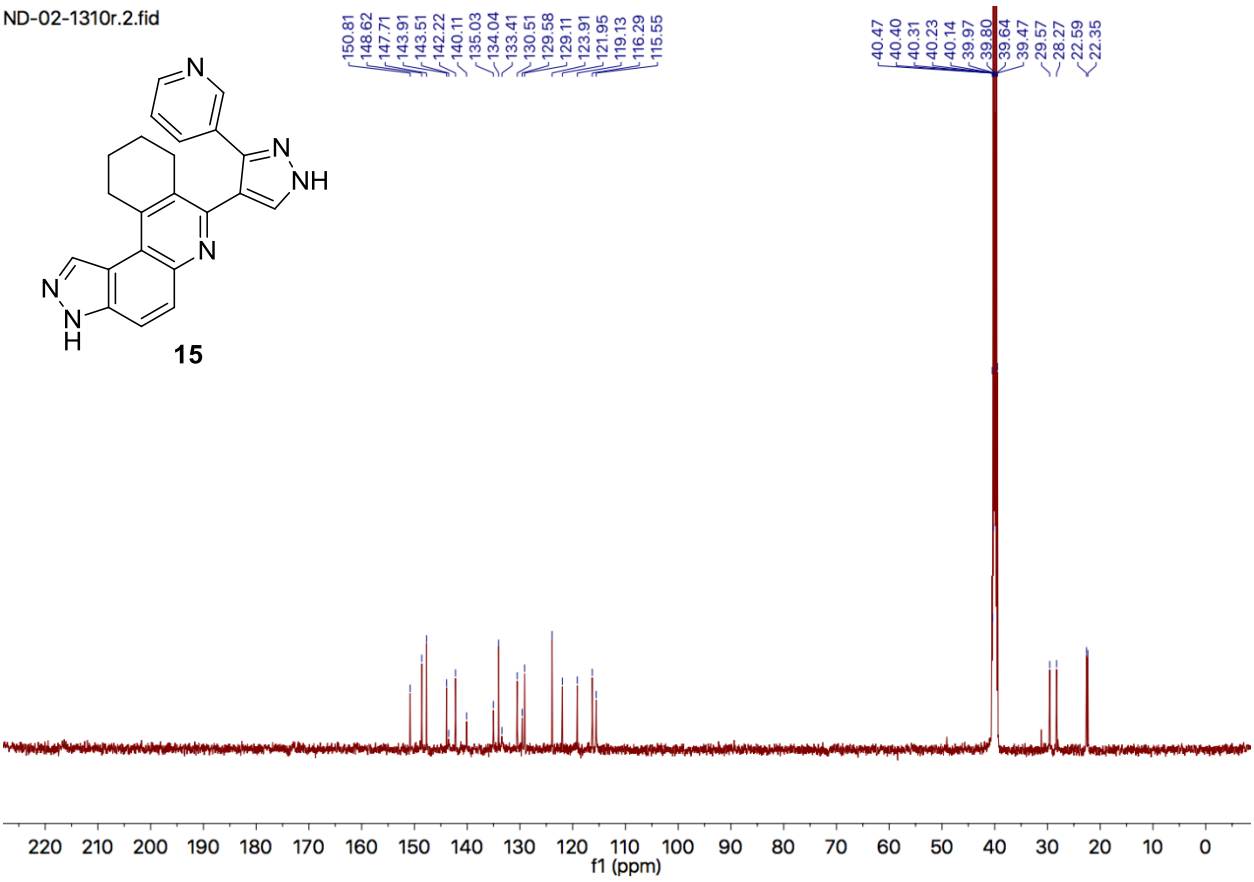
^{13}C NMR of compound **14**



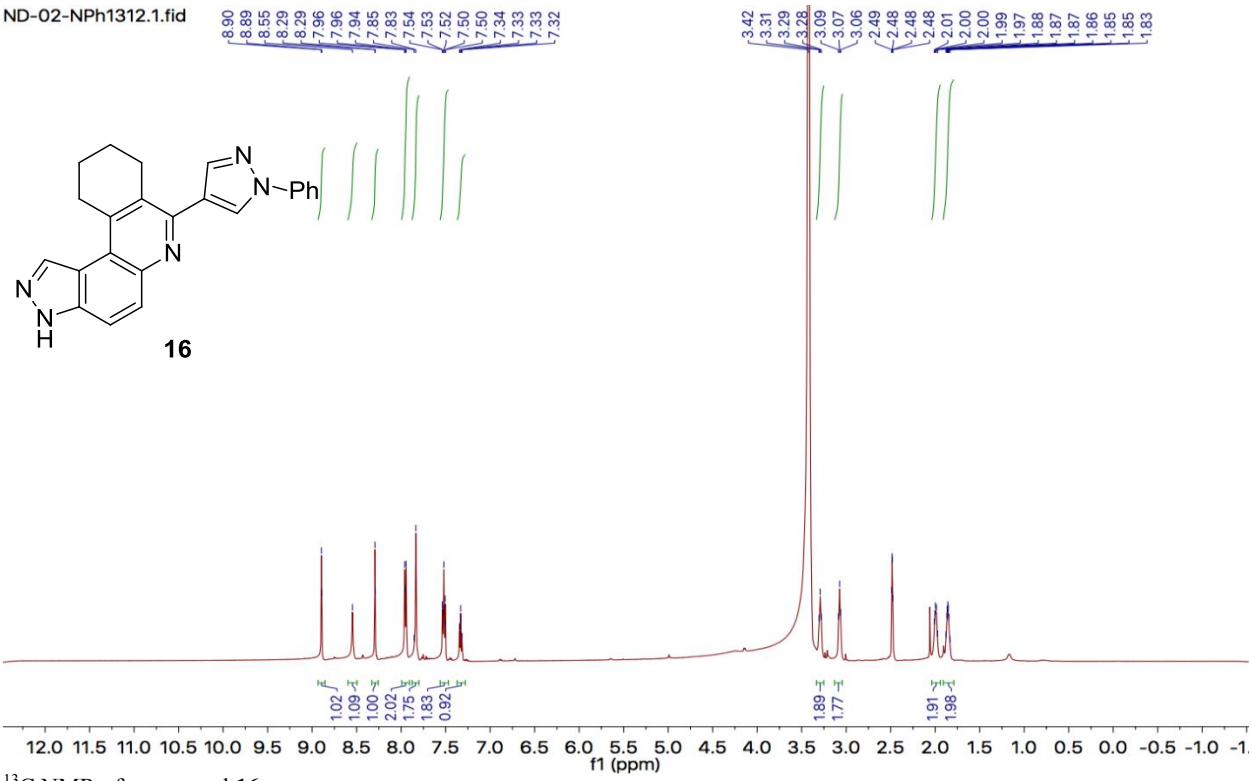
^{1}H NMR of compound **15**



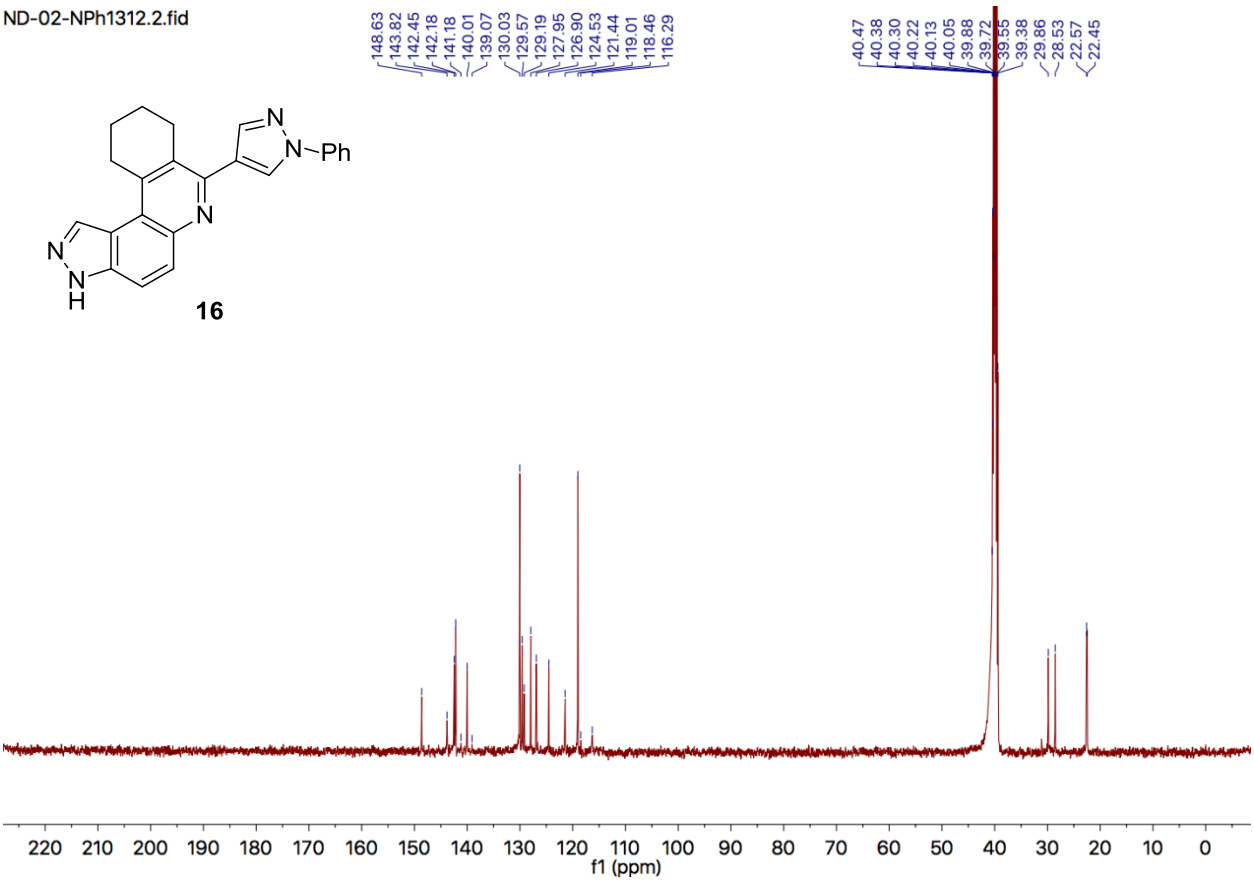
^{13}C NMR of compound **15**



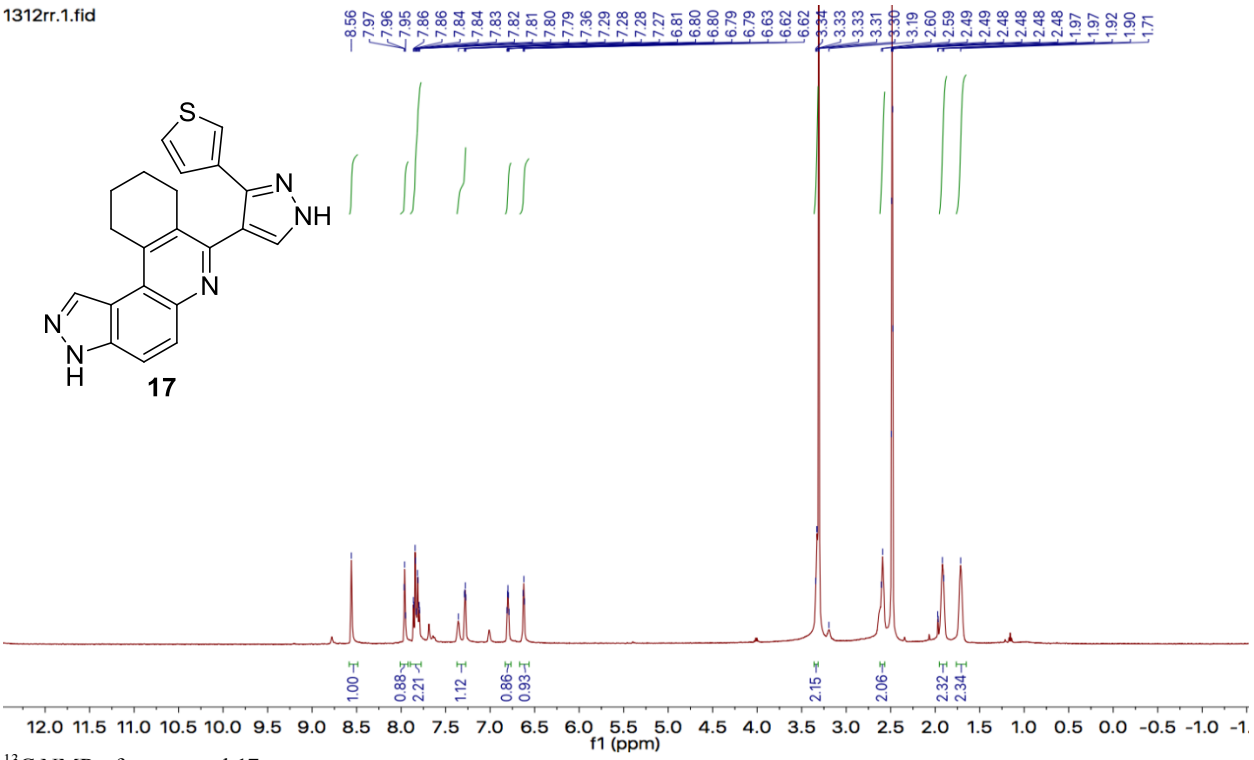
^{1}H NMR of compound **16**



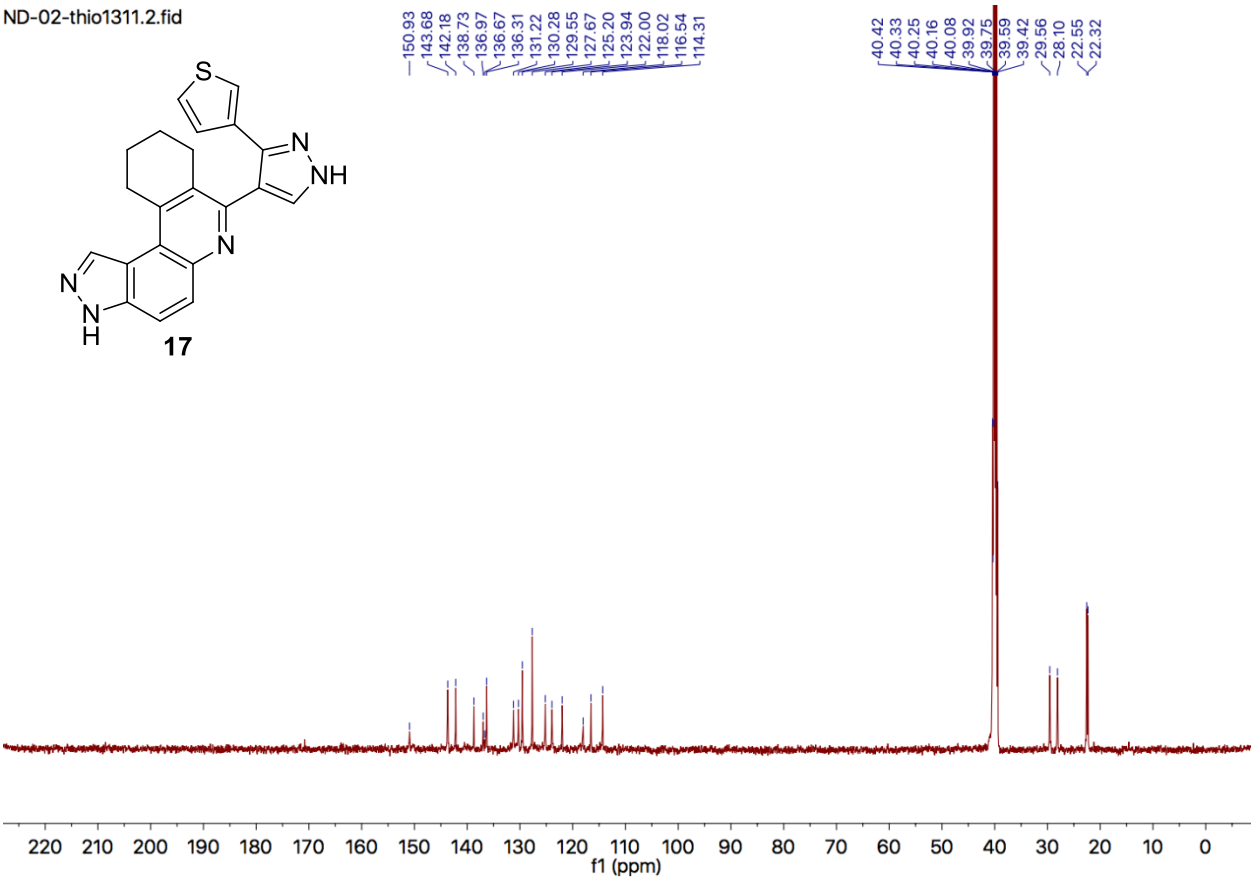
^{13}C NMR of compound **16**



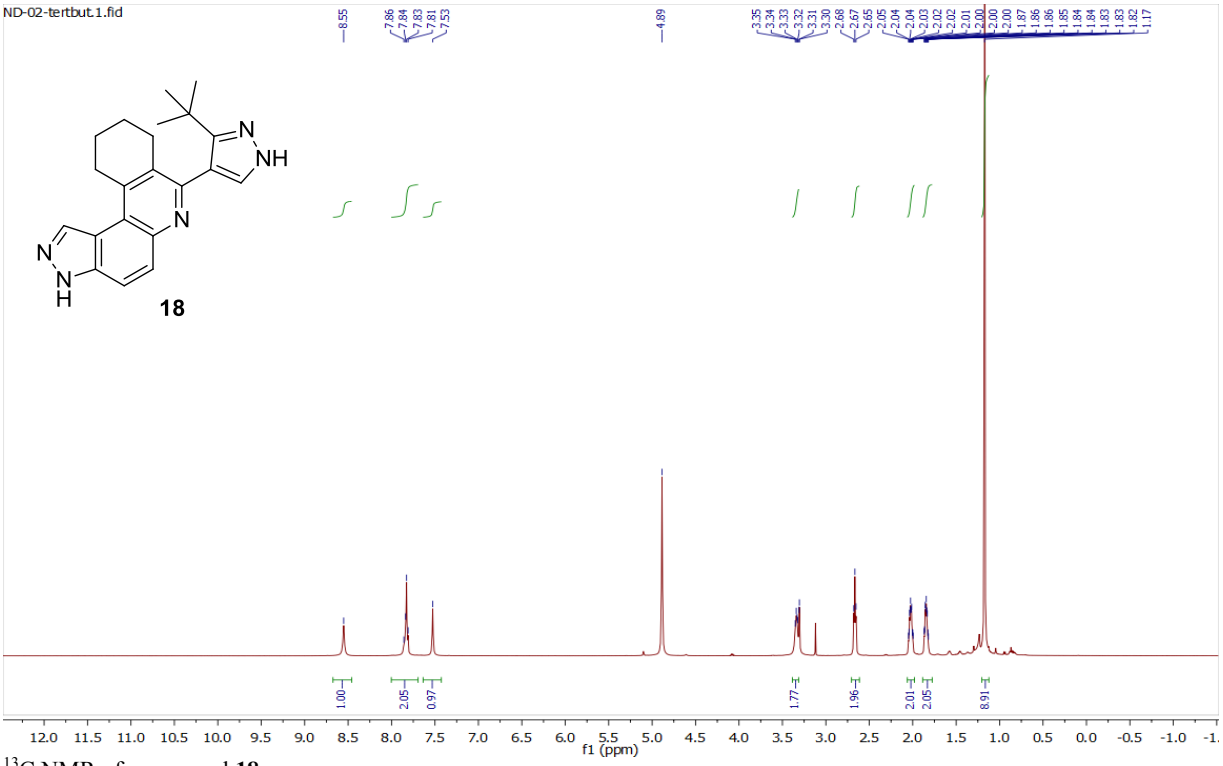
^{1}H NMR of compound **17**



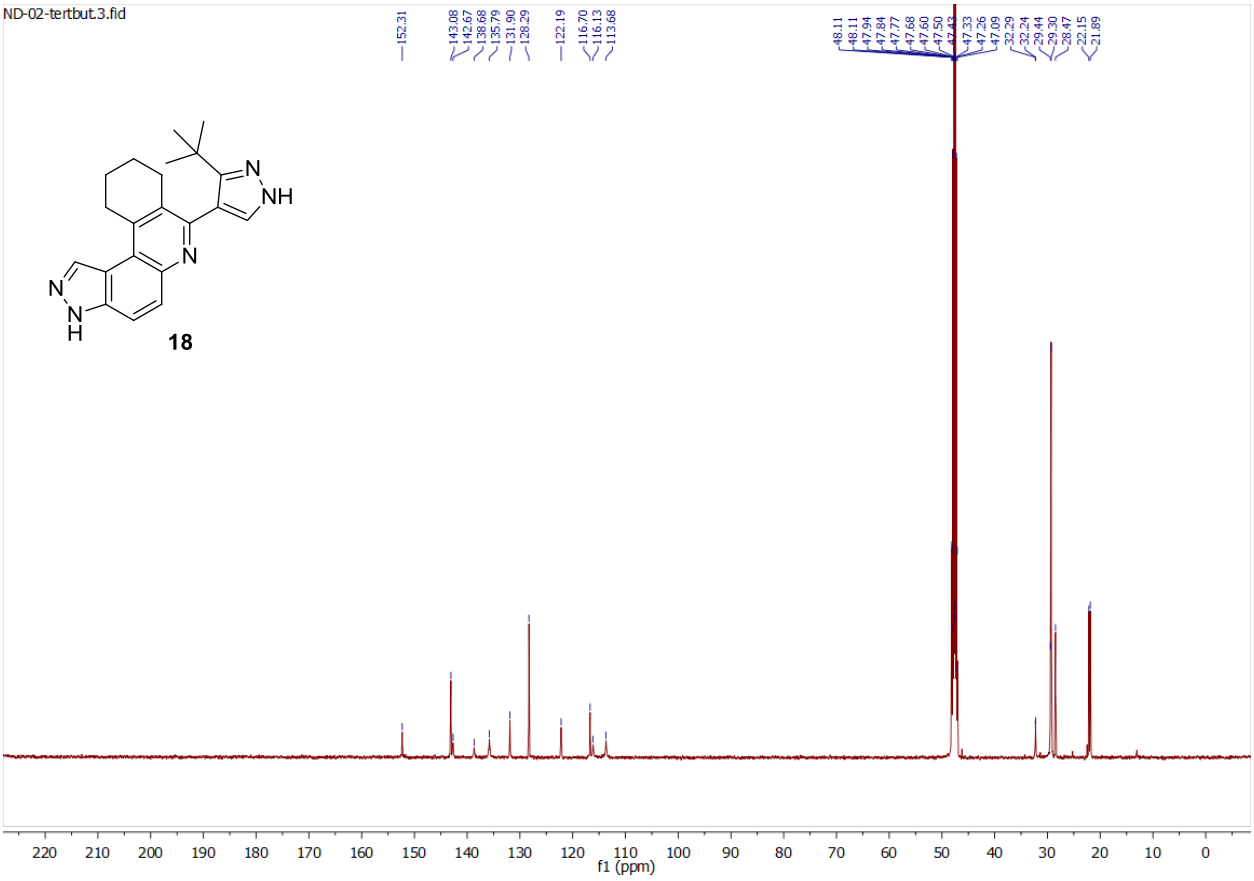
^{13}C NMR of compound **17**



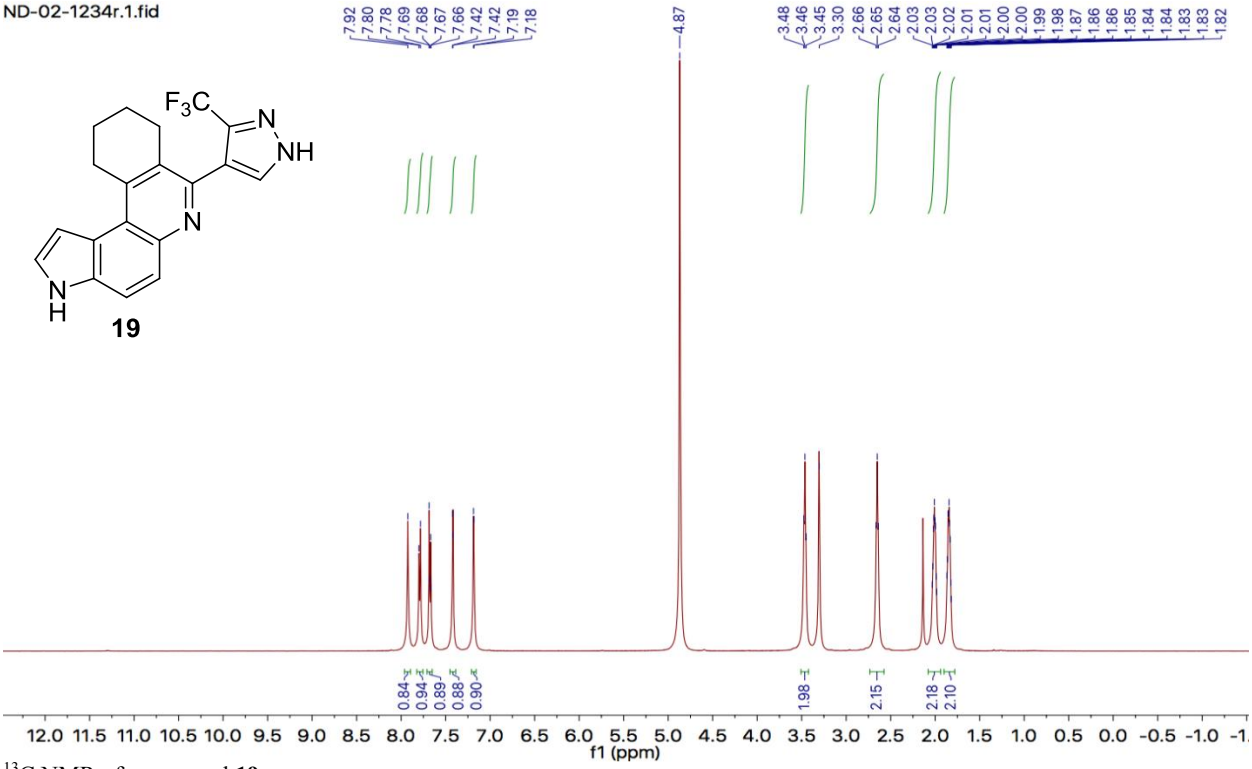
^{1}H NMR of compound **18**



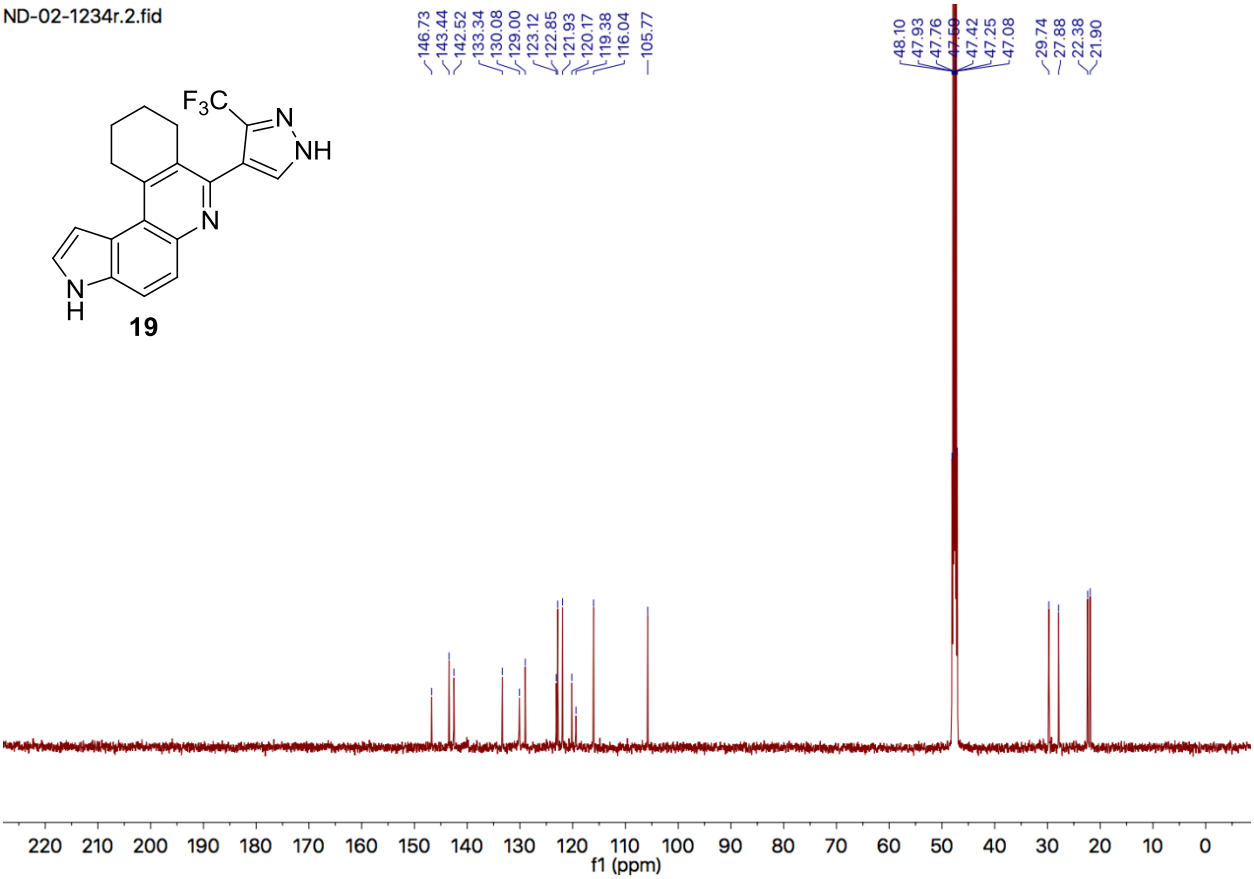
^{13}C NMR of compound **18**



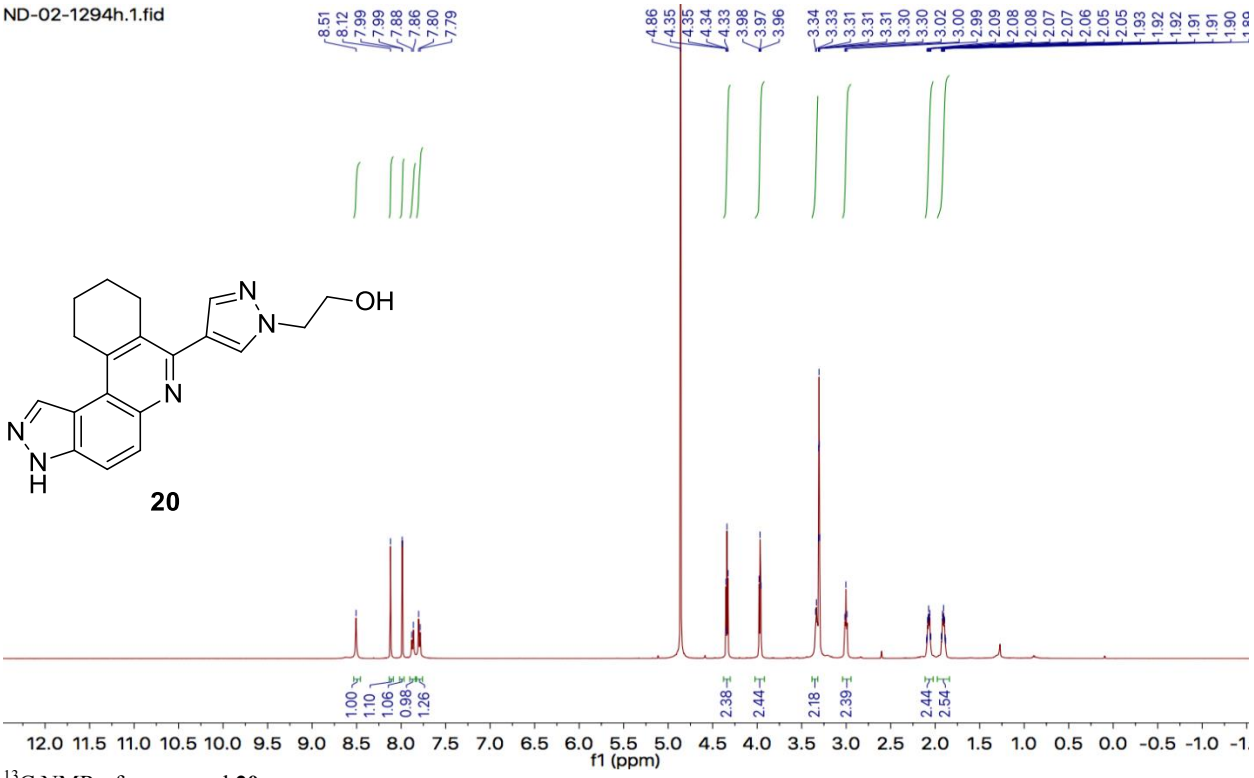
^{1}H NMR of compound **19**



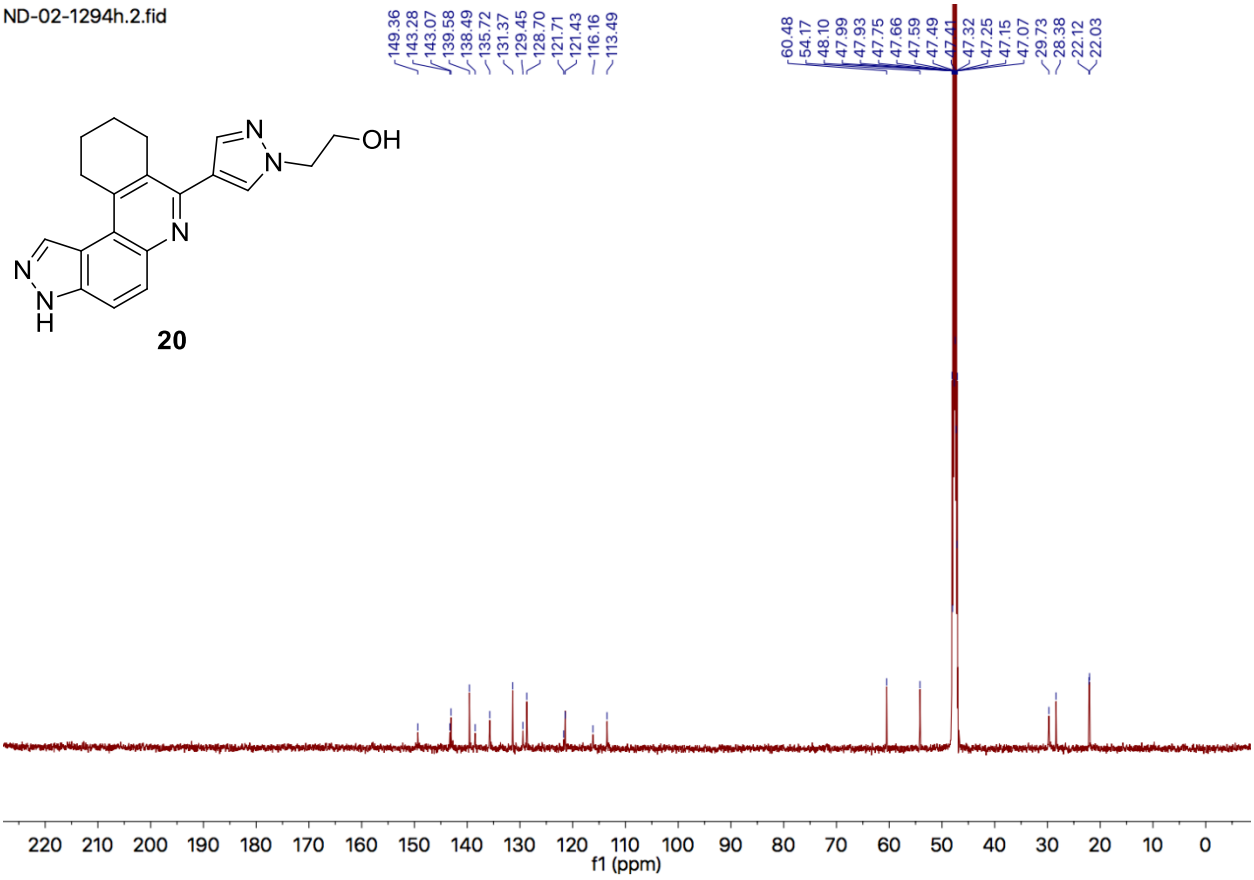
^{13}C NMR of compound **19**



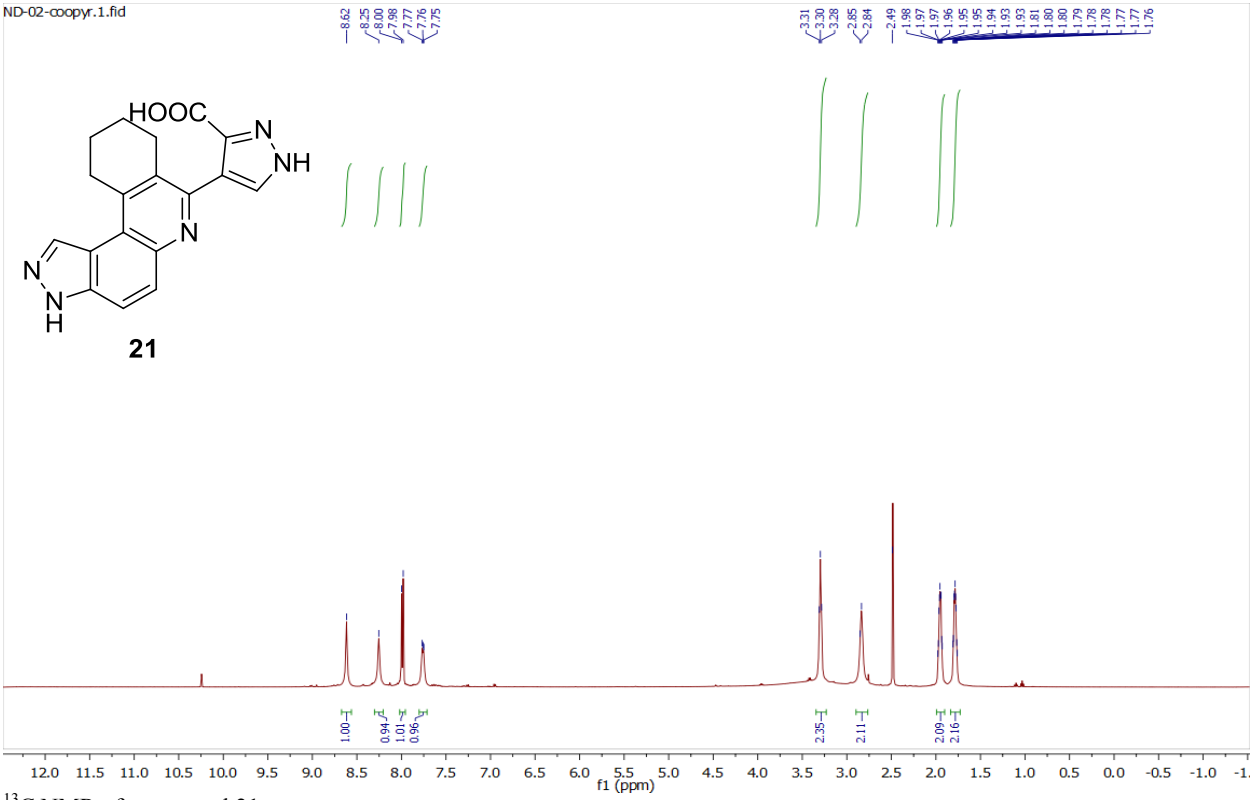
^{1}H NMR of compound **20**



^{13}C NMR of compound **20**



^{1}H NMR of compound **21**



^{13}C NMR of compound **21**